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Research Article

Applying flukeprint environmental DNA to detect skin-associated bacteria from free-ranging West Coast Transient killer whales *Orcinus orca rectipinnus*

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Abstract

Globally, vessel strikes, entanglements, and ocean pollution negatively impact cetaceans by compromising skin integrity and health through direct injury or disease and by increasing susceptibility to chronic bacterial, viral, or fungal infections. Characterizing skin microbial assemblages in free-living cetaceans offers a means to evaluate and establish baseline trends in skin conditions. Currently, skin microbial data are collected only via biopsy sampling, which is a mildly invasive approach that represents only a small surface area of skin. In this study, we employed non-invasive environmental DNA (eDNA) flukeprint sampling of West Coast Transient killer whales in California and British Columbia to evaluate the method's effectiveness in detecting unique skin-associated bacteria. Using a paired approach, we found a greater number of bacterial exact sequence variants and bacterial genera unique to flukeprints compared with seawater controls. Functional pathways of unique flukeprint bacterial exact sequence variants were dominated by predicted functions likely related to DNA replication and repair, cell signaling, and protein folding. We compared bacterial results with visual skin condition assessments and other factors, finding that flukeprint bacteria are explained by a combination of geographic region, sampling month, whale age, whale sex, and the percentage of skin lesions visible in lateral photographs. This work demonstrates the applicability of eDNA flukeprint sampling for improving understanding of cetacean skin health, with implications for studying endangered or surface-elusive cetaceans that are not good candidates for biopsy sampling.

Key words: eDNA, flukeprints, killer whale, scarring, skin lesions, West Coast Transient, whale conservation

Introduction

Species of cetaceans worldwide are under increasing pressure from anthropogenic activities (Bressem et al. 2009a; Avila et al. 2018; Nisi et al. 2024). Killer whales (*Orcinus orca*) are found in all the world's oceans and have varying distributions, prey preferences, and behaviors depending on their population of origin (Wade and Forney 2007; de Bruyn et al. 2013; Morin et al. 2015). In the northeast Pacific, mammal-eating West Coast Transient (or Bigg's) killer

whales (hereafter WCT) occupy the largest range of the North Pacific killer whale ecotypes, extending from Alaska in the north to California in the south (Morin et al. 2024). The inland waters of southern British Columbia (B.C., Canada) and Washington State (U.S.), commonly referred to as the Salish Sea, are frequented year-round by WCT (Houghton et al. 2015; Shields et al. 2018). This pattern is likely due to the high prevalence of WCT-preferred prey species, harbor seals (*Phoca vitulina*; Fisheries and Oceans Canada 2013), which have remained stable in number since the mid-1990s (Fisheries and Oceans Canada 2019), following government protections enacted in the 1970s (Shields et al. 2018; Fisheries and Oceans Canada 2024). Off the coast of central California, the region surrounding Monterey Bay and Big Sur is also an important area for WCT. Here, WCT are frequently encountered preying on migrating gray whale calves between April and May and on sea lions throughout the summer and fall months (Black et al. 2023). The predatory niche of this killer whale ecotype means that they are commonly found in nearshore coastal waters, where human activity is concentrated, including vessel, industrial, and commercial activities (Small and Nicholls 2003; Crain et al. 2009; Cloern et al. 2016).

Anthropogenic trauma such as vessel strikes, entanglement in fishing gear, and ocean pollution can cause skin damage and skin disease in killer whale populations, with skin condition serving as a major indicator of health for many cetacean species (Bressem et al. 2009a, 2009b; Mouton and Botha 2012; Apprill et al. 2014; Clegg and Butterworth 2017). Susceptibility of individuals and populations to infectious agents can be increased through stress caused by factors including pollution, reduced prey availability, and general disturbance (Ross 2002; Martineau 2007; Beineke et al. 2010; Koch et al. 2018). For killer whales, skin lesions have previously been observed to contain unclassified poxviruses, papillomaviruses, and other unknown causal agents (Bossart et al. 1996; Gaydos et al. 2023). While visual assessment of skin integrity can be performed by analyzing lateral and drone-based images (Hamilton and Marx 2005; Bertulli et al. 2012; Herr et al. 2020; Gaydos et al. 2023), characterizing skin microbiomes typically requires bacteriological sampling via swabs for captive or deceased cetaceans (Raverty et al. 2014, 2020; Chiarello et al. 2017; Dominguez-Sanchez et al. 2023) or the collection of skin biopsies from free-living cetaceans (Apprill et al. 2014; Bierlich et al. 2018; Hooper et al. 2019; Van Cise et al. 2020; Toro et al. 2021; Jia et al. 2022).

To mitigate research impacts on at-risk populations, less invasive methods are being developed and applied to obtain information on cetacean microbiomes and population genetics. The collection of cetacean "blow," or exhaled breath, via either direct sampling (i.e., poles and petri dishes; Hogg et al. 2009; Acevedo-Whitehouse et al. 2010; Raverty et al. 2017; Robinson and Nuuttila 2020; Vendl et al. 2020; Rhodes et al. 2022) or drones (Apprill et al. 2017; Pirotta et al. 2017; Centelleghe et al. 2020; O'Mahony et al. 2024; Santos et al. 2024) enables the collection of genetic data from free-living cetaceans. Environmental DNA (eDNA), referring to seawater collected within the close vicinity of cetaceans, has also been applied to obtain individual-level genetic data (Suarez-Bregua et al. 2022). Flukeprints, defined as the disturbed patch of water produced as an individual whale dives, have proven to be a useful source of DNA not only for species detection (Foote et al. 2012; Baker et al. 2018, 2023; Alter et al. 2022; Robinson et al. 2024; Rodriguez et al. 2025), but

also for population genetic assessments (Parsons et al. 2018, 2024; Székely et al. 2021; Bennington et al. 2024) and the collection of demographic data such as sex (Robinson et al. 2025b). Despite detectable cetacean flukeprint eDNA being primarily sourced from sloughed skin cells (Suarez-Bregua et al. 2022), there has been no prior application of eDNA for detecting and characterizing skin microbiomes of free-living cetaceans.

In this study, we aimed to (1) determine whether unique WCT bacteria can be detected from flukeprint eDNA samples; (2) determine whether bacteria detected uniquely in flukeprints are likely skin-associated through functional pathway assessment; and (3) evaluate the relationship between bacteria detected in flukeprint samples and individual whale skin integrity using lateral image assessments.

Methods

The following methods were carried out in accordance with relevant guidelines and regulations. A permit was not required to collect seawater samples in Monterey Bay, as the project did not fall under the requirements for a directed take permit issued under the Endangered Species Act or the Marine Mammal Protection Act. For B.C. research, all samples were collected under Marine Mammal Licence MML18, issued by Fisheries and Oceans Canada (DFO). This study did not involve direct experimentation on or handling of whales. All data were collected using non-invasive methods (e.g., environmental DNA sampling and visual observation). As no animals were captured, restrained, or manipulated in any way, approval from an animal ethics committee was not required. All guidelines and conditions outlined in the DFO permit were strictly followed. Informed consent was obtained from all participants or their legal guardians prior to participation in sample collection and photography.

Sample collection

Flukeprint eDNA samples were opportunistically collected within both the Monterey Bay region (California, U.S.) and the southern Salish Sea (B.C., Canada) between January and November 2024 (Fig. 1). Upon encountering WCT, a target individual whale was approached from a parallel position in a motorized research vessel, maintaining an approximate distance of 100 m until a terminal dive was observed. After the individual had dived, the research vessel maneuvered immediately beside the flukeprint for collection, taking care not to cross in front of the last observed direction of travel of the individual. Flukeprint eDNA samples were collected from individual whales 30 s to 1 min after a terminal dive (Robinson et al. 2025a). Paired lateral images were collected prior to or following sample collection to confirm identities, sexes, and ages of sampled individuals using published photo-identification catalogues (Towers et al. 2019; California Killer Whale Project 2023). Both left and right photo-identification images were taken, where possible, following standard image protocols for killer whales in the Pacific (Towers et al. 2019).

Samples were collected onboard Point Sur Clipper (13.7 m length), Sea Wolf II (19.8 m length), a RHIB (6.7 m length), and R.V. Skana (8.3 m length). Due to variation in vessel freeboards (80 cm on Skana compared with between 142 and 160 cm on Point Sur Clipper and Sea Wolf II), samples were either collected

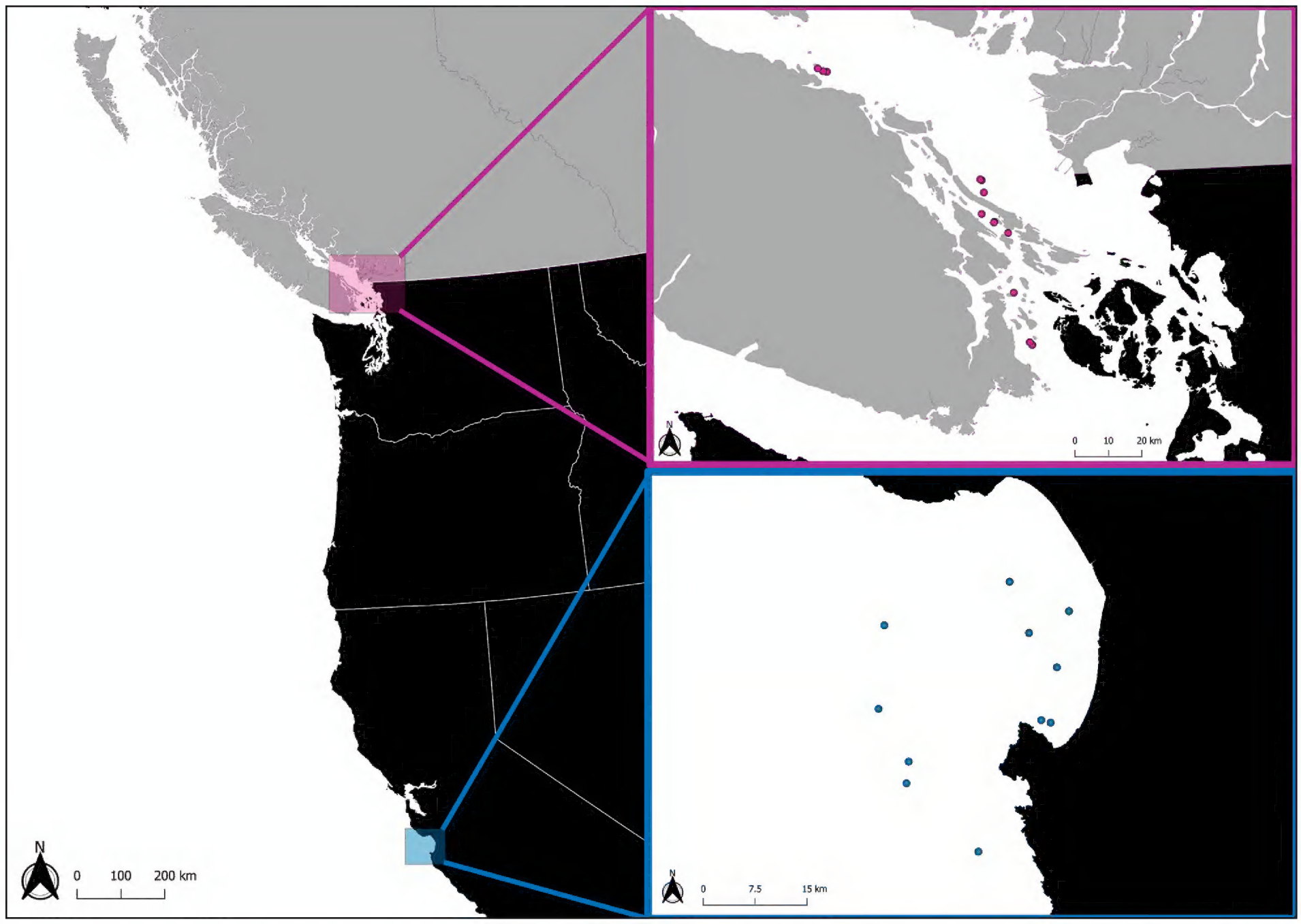

Figure 1. Map of environmental DNA (eDNA) samples collected in the study. Samples were collected from West Coast Transient killer whales (*Orcinus orca*) in British Columbia (purple) and California (blue). The purple-outlined map refers to the Canadian study site (n = 13), and the blue-outlined map refers to the United States study site (n = 11). Map source: QGIS (v3.18; www.qgis.org) using Coordinate Reference System EPSG:26910—NAD83 / UTM zone 10N—projected.

using a previously sterilized 5 L bucket (15% bleach solution followed by a distilled water rinse) attached to 3 m of line via marine-grade sterilized chains for large-vessel-collected samples (i.e., Point Sur Clipper and Sea Wolf II) or using a previously sterilized 3.75 L jug, scooped by hand, for samples collected from smaller vessels (i.e., Skana and RHIB; Suppl. material 1: fig. S1). All samples were collected only under conditions resembling < 4 on the Beaufort Scale (Robinson et al. 2024) to ensure that flukeprints were visible for collection.

Two liters of each resulting eDNA sample were filtered within three hours of collection through a Citizen Science Sampler (Smith-Root, Inc.) using 0.45 µm self-preserving filters (Smith-Root, Inc.). If samples could not be immediately filtered after collection, buckets or jugs were sealed with sterilized lids and stored in a dark location onboard the vessel. Metadata, including the date and time of sample collection, GPS coordinates, individual whale alphanumeric designation, and photograph frame number to be later matched to respective photo-identification catalogues (Towers et al. 2019; California Killer Whale Project 2023), as well as any relevant sample collection notes, were recorded for each encounter. For each whale encounter from which flukeprint(s) were collected, a seawater control sample (2 L) was also collected to characterize the local baseline bacterial community. In encounters where multiple flukeprint samples were obtained within the same surface water mass (i.e., within 500 m of one

another), a single seawater control was collected due to the likely high autocorrelation of microbial taxa at the spatial and temporal scales of the encounter (Needham et al. 2013; Weber and Apprill 2020). This approach follows previous biopsy-based microbiome studies, which similarly did not always apply a 1:1 control-to-biological sample ratio (Apprill et al. 2011a; Jia et al. 2022). All seawater controls were collected adjacent to the immediate area through which whales had passed after the encounter ended and were processed in the same manner as flukeprint samples. All sample collection and filtering were conducted while wearing sterile nitrile gloves and following best practices to avoid DNA contamination. All filters were labeled appropriately and stored in a cool, dark location before shipment to the Pacific Science Enterprise Centre (PSEC) in Vancouver (B.C., Canada), where they were stored at −20 °C prior to further analyses.

DNA extraction, library preparation, and sequencing

Prior to and after sample processing, all surfaces within a dedicated eDNA room were thoroughly decontaminated using 10% bleach and 80% ethanol solutions. All extraction tools were also sterilized using 10% bleach, UltraPure™ DEPC-treated water (Invitrogen™), and 80% ethanol rinses. All filters were extracted using the DNeasy® Blood & Tissue Kit (Qiagen) in the eDNA room following a modified version of the DNeasy® extraction protocol (Qiagen 2020), with a reduced final elution volume. Filters were first cut in half and then chopped into pieces ~3 mm in diameter before being placed into two 1.5 mL tubes, each containing 300 µL Buffer ATL and 340 µL phosphate-buffered saline (PBS). Filter pieces were divided into two tubes per sample to maximize efficiency of the lysis step. A sterile metal bead was added to each sample tube before samples were shaken at 30.0 Hz for 45 s in a Retsch® MM 400 Mixer Mill. Next, 30 µL of proteinase K was added to the samples prior to overnight incubation at 56 °C in a rotating hybridization oven. After incubation, the tubes were centrifuged at 17,000 × g for 1 min, and the resulting supernatant was transferred to a new tube. During the spin column step, supernatants from both sample tubes were passed through a single spin column, combining them into one extract for subsequent steps. Finally, eDNA was eluted using 50 µL Buffer AE. Extractions were conducted in three batches, and for each batch, a negative extraction control consisting of no filter was processed. DNA purity was measured using a NanoDrop™ 2000 Spectrophotometer, and sample concentration was measured using a Qubit 4 Fluorometer (Invitrogen™) with a High Sensitivity Qubit™ dsDNA Quantification Assay Kit. All extraction blanks had DNA concentrations below the detection threshold of the high-sensitivity assay. DNA extracts and negative controls were then stored at −20 °C until further processing.

16S amplification, library preparation, and sequencing were carried out at the High Throughput Biology Facility (Biofactorial) in the Life Sciences Centre at the University of British Columbia (Vancouver, B.C., Canada). All eDNA samples, negative seawater controls, and extraction blanks were amplified following PCR setup as detailed in Comeau et al. (2011, 2017). Briefly, a dual-indexing, one-step 10 µL PCR reaction was performed on a LabCyte Access Workstation using Quanta repliQa HiFi ToughMix with 1 ng input DNA and complete fusion primers that include Illumina Nextera adaptors, indices, and regions targeting the V4/V5 region of the 16S rRNA genes (Comeau et al. 2017). One extraction blank was spiked

with a positive control consisting of a ZymoBIOMICS Microbial Community DNA Standard (cat. no. D6305; Zymo Research). Amplicons were quantified using a PicoGreen assay (Quant-iT™ PicoGreen™ dsDNA Assay Kit, Thermo Fisher), and 2 ng of each product was pooled for subsequent cleanup using the AMPure XP PCR cleanup protocol (Beckman). The pooled library was quantified using a PicoGreen assay and loaded onto an Illumina NextSeq XLEAP P1 600 following the manufacturer's recommendations. Samples that failed to provide adequate libraries were subsequently subjected to a 1:10 dilution to reduce potential PCR-inhibiting compounds prior to library re-preparation. Samples that failed to provide adequate libraries after dilution were excluded from sequencing.

Bioinformatic analysis

Resulting paired-end reads were processed using the MetaWorks v1.9.3 multi-marker metabarcode pipeline, available from https://github.com/terrimporter/MetaWorks, using the standard exact sequence variant (ESV) workflow (Porter and Hajibabaei 2022). This pipeline was selected due to its incorporation of Snakemake for efficient computational resource management, pseudogene filtering options, and prior application in eDNA studies across different ecosystems, including freshwater and soil systems (Porter and Hajibabaei 2022; Robinson et al. 2022; Milián-García et al. 2023). Reads were paired using SEQPREP v1.3.2 (St John 2016) with default parameters, except that the Phred score quality cutoff was set to 20 and the minimum overlap was set to 25 bp. CUTADAPT v3.2 (Martin 2011) was then used to trim primers using default parameters, except that the minimum sequence length was set to 150 bp, Phred quality score cutoffs at read ends were set to 20, and the maximum number of Ns was set to 3. Dereplication and denoising were performed using VSEARCH v2.15.2 (Rognes et al. 2016) following the MetaWorks UNOISE-style workflow. Exact-sequence dereplication was applied using default exact-match parameters. Denoising was performed using the --cluster_unoise approach with a minimum cluster size of 3 (--minsize 3), removing singletons and doubletons while applying the default UNOISE error model for sequence correction. De novo chimera detection was conducted using VSEARCH's uchime_denovo algorithm. The --minsize 3 threshold was selected to reduce spurious sequences arising from stochastic sequencing errors in low-concentration eDNA samples (e.g., flukeprints and seawater controls) while retaining true biological diversity (Flynn et al. 2015). Resulting reads were taxonomically assigned using the RDP Classifier v2.14 (Wang et al. 2007) with the built-in 16S reference set, and a minimum bootstrap support value of 0.80 was applied at each taxonomic rank. The RDP Classifier was selected because it provides a stable and reproducible framework for ESV classification, which is suitable for comparative analyses even when full community resolution is not achieved (Wang and Cole 2024). This classifier has been applied in other eDNA-based microbiome studies (Tessler et al. 2017; Farrell et al. 2018; dos Santos and Blabolil 2025) and shows high concordance with additional databases, including Greengenes, SILVA, and NCBI (Balvočiūtė and Huson 2017). Samples with fewer than 1,000 reads were discarded, and all unclassified reads were excluded prior to further analysis. This approach is commonly used in DNA metabarcoding studies of low-quality environmental samples (e.g., García-Machado et al. 2023; Dan et al. 2024)

to ensure adequate sequencing depth and reduce potential artifacts. All ESVs detected in extraction blanks were removed from the dataset prior to rarefaction and further analyses, as described in previous eDNA-based metabarcoding studies (e.g., Jeunen et al. 2022; Lavrador et al. 2024; Sevellec et al. 2025).

Image evaluation and scoring

Photo-identification images of individuals whose flukeprint samples contained unique bacteria (i.e., distinct from surrounding seawater samples) were sorted and evaluated for quality to assess skin integrity. Highest-quality images were defined as those taken when the vessel was directly parallel to the individual, with the dorsal fin and saddle patch clearly visible, similar to protocols described in other skin assessment studies (Grimes et al. 2022; van Weelden et al. 2025). To ensure skin integrity was accurately assessed for each individual, one high-quality image from the left dorsal side and one from the right were required. If both a left and a right image were not taken on the same day as the eDNA sample, or if no high-quality dorsal image was available, additional images were curated from the online platform *Finwave* (https://finwave.io/), a public photo-identification database for killer whale populations. Images taken within 150 days of the sampling event were considered for analysis to coincide with the healing rates of small open wounds (Dwyer and Visser 2011) in killer whales, as scars remain for multiple years (van Weelden et al. 2025), and lesions are often associated with rake scars, with no apparent seasonal occurrence patterns (Gaydos et al. 2023).

Left and right images were standardized for skin assessments by aligning the dorsal fin along the horizontal plane using the straightening tool in ACDSee Photo Studio 7 software (v7.0.1840). This alignment allowed the visible surface area (VSA), defined as the portion of the body region not obscured by water, of each respective side to be divided into four regions. Although images met the criteria for photo identification, factors such as glare, water obscuration, focus, and the extent of the whale visible may still influence skin assessments (Corsi et al. 2022; Grimes et al. 2022; van Weelden et al. 2025). To account for this potential variability, each of the four body regions was scored from 1 (low quality) to 3 (high quality; see definitions and examples in Suppl. material 1: table S1). All regions were assessed for skin integrity regardless of their regional score, except for low-quality regions (score 1) where countershading, blurriness, or ocean spray hindered the ability to visually detect skin anomalies.

Measuring skin lesions and scarring

The software ImageJ (v1.53) was used to systematically divide lateral images into body regions and measure skin integrity. Rotated lateral images were divided into four distinct body regions: (1) dorsal fin, (2) anterior, (3) mid-flank, and (4) peduncle (Nicholls et al. 2023; Suppl. material 1: fig. S2). These regions are frequently exposed during killer whale surfacing patterns and have been used to examine skin abnormalities, such as lesions and scars, in killer whale populations (Grimes et al. 2022; Gaydos et al. 2023). The line tool was used to draw a horizontal line across the base of the dorsal fin, starting above the posterior edge of the saddle patch. Two vertical lines were then drawn at either end of the horizontal line, separating the dorsal fin into one region and the body into three distinct regions.

Skin lesions were assessed using a percent-coverage approach (Corsi et al. 2022). The VSA was outlined using the polygon tool and measured to determine the total area of each region in pixels (Suppl. material 1: fig. S3). Skin lesions were classified as (1) gray patches, (2) gray targets, (3) pinpoint black discoloration, (4) erosions, or (5) cephalopod lesions (Gaydos et al. 2023). All lesions were measured for area using the polygon tool, and their pixel counts were summed to obtain the total lesion area per region per lateral image. After image measurements were completed, each whale was assigned a skin abnormality presence score (1: None, 2: Possibly, 3: Yes) for each of the five lesion categories, as well as for any evidence of open or partially healed wounds.

In addition to skin lesions, a scar index was estimated for the VSA of each region using four categories: (1) None—no scarring in the region; (2) Mild—less than 25% of the region covered by scars; (3) Moderate—between 25% and 50% of the region covered by scars; and (4) Severe—greater than 50% of the region covered by scars (Robeck et al. 2019). Contrary to a previous study (Robeck et al. 2019), all scars, including singular lines, were recognized, as any breakage of the dermal layer may introduce bacteria (Mouton and Botha 2012).

To mitigate observer bias, skin assessments were conducted by two observers. Pixel counts for total regional area and lesion area, types of skin abnormalities present, and scar index were averaged between observers. These averaged measurements were then used to assess the influence of skin variables on total bacterial ESVs and total bacterial genera detected in sampled flukeprints.

Data analyses and visualizations

Data were analyzed in RStudio v2024.09.0 (RStudio 2023) using R 4.0.3 (R Core Team 2024) on a Linux Ubuntu 20.04 system (Sobell 2011).

eDNA analyses

Rarefaction curves were first visualized using the *rarecurve* function from the *vegan* package to assess sequencing depth across samples, and samples were subsequently rarefied to equal depth using the *rrarefy* function prior to downstream analyses. Although rarefying has been criticized (e.g., McMurdie and Holmes 2014), recent studies demonstrate that rarefaction is appropriate for data normalization under conditions of uneven library size, small or highly variable read depth, and when using presence/absence or nonparametric community metrics (Weiss et al. 2017; Hong et al. 2022; Schloss 2024). In particular, rarefying helps prevent library-size bias in beta-diversity analyses and maintains appropriate Type I error control in permutation-based tests. In recent studies of marine eDNA metabarcoding, rarefaction has been widely applied as a standard step in analysis pipelines (e.g., Dully et al. 2021; Shirazi et al. 2021; Pascoal et al. 2023). Next, to test for significant differences, we assessed normality using visual methods (*ggdensity* and *ggqqplot* functions in R) and the Shapiro–Wilk test (Shapiro and Wilk 1965).

Diversity analyses were conducted using the *vegan* package (Oksanen et al. 2001). We calculated ESV richness across different partitions of the data to compare differences among sample types (i.e., flukeprint or seawater control) and sampling locations (i.e., California or B.C.). Venn diagrams

were generated using the *VennDiagram* package (Chen 2022) to visualize the total number of bacterial genera found exclusively in flukeprint samples (hereafter referred to as "unique") or shared between flukeprint and blank samples, and to assess overlap between paired flukeprint and seawater control samples. To evaluate the most common bacterial taxa detected across both sample types, we plotted the top 30 genera by ESV count. Community structure was evaluated using two complementary ordination approaches. Non-metric multidimensional scaling (NMDS) was performed on Bray–Curtis dissimilarities using the *metaMDS* function in *vegan* to visualize differences among sample types, locations, and individual whales. Two dimensions were selected following assessment with the *dimcheckNMDS* function from the *goeveg* v0.5.1 package (von Lampe and Schellenberg 2024). Stress values were assessed using the *stressplot* function, and dispersion among groups was evaluated using *betadisper* and the base R *anova* function. We used PERMANOVA and the *adonis2* function and 999 permutations to test for significant interactions among groups (sample type, collection location, WCT sex, and WCT age). Principal coordinates analysis (PCoA) was conducted on Bray–Curtis distances using the *cmdscale* function to quantify variance explained along principal axes. Differences among sample types, collection locations, and individual whales were tested using PERMANOVA with the *adonis2* function (999 permutations).

We visualized the frequency of unique ESVs detected from bacterial families unique to flukeprints using a heatmap generated with the *geom_tile* function from the *ggplot2* package (Wickham et al. 2024). To explore contributions of seawater controls to flukeprint samples, we applied the *FEAST* package (Fast Expectation-mAximization microbial Source Tracking; Shenhav et al. 2019) within each geographic location. For each location (California and B.C.), count matrices of bacterial genera were prepared, and corresponding metadata files were generated to align seawater control sources with each sink. FEAST was run with the *different_sources_flag* set to zero to allow contributions from all seawater controls to each sink (i.e., flukeprint sample). FEAST outputs were visualized using *ggplot2* (Wickham et al. 2024). Functional pathway abundances were predicted from 16S rRNA ESVs unique to flukeprint samples using PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) v2.5.1 (Douglas et al. 2020). A subset of the top 20 enzymes and pathways was assessed using MetaCyc (Caspi et al. 2018), a curated database of metabolic pathways, to identify the most likely source of each enzyme and pathway based on known microbial contributions. Enzyme and metabolic pathway abundances were used for downstream analyses, including identification of likely associations (i.e., skin, seawater, or general pathways) by combining MetaCyc annotations with comparisons to previously published cetacean skin microbiome pathway associations. Bar plots of the most abundant enzymes were generated and color-coded by major class (i.e., oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases). Heatmaps were generated using the *pheatmap* package (Kolde 2025) to visualize the relative abundance of the top 20 pathways. Abundance values were log-transformed and row-scaled to emphasize relative differences among samples within each pathway. Pathways were annotated by skin association, and hierarchical clustering was applied to samples.

Skin integrity analyses

Flukeprint sampling dates were converted to Julian dates to calculate the number of days between collection of flukeprint samples and lateral images. Images outside the 150-day threshold were removed. Stacked bar charts were created using *ggplot2* (Wickham et al. 2024) to visualize the percentage of lesions and scarring of the VSA for each of the four body regions and to compare results among individuals and between locations. The total scar index was converted to a relative frequency per region and categorized as a factor (1 = None; 1 > Mild ≤ 2.25; 2.25 > Moderate ≤ 3.25; Severe > 3.25) to enhance visualization. The skin abnormality presence score was also converted to a factor (1 = None; 1.5–2 = Possibly; 2.5–3 = Yes) and summarized per body region. Violin and box plots (*ggplot2* package) were used to visualize mean percentages of VSA with lesions among whales, and a permutation test with 10,000 iterations was performed to assess statistical significance of observed differences.

We then tested the effect of region score on percentage of lesions and scar index using ANOVA (Kaufmann and Schering 2014). First, the percentage of lesions in each region was calculated by dividing the observer-averaged total lesion area (in pixels) by the total area of the region (in pixels) and multiplying by 100. Next, the *aov* function was used to assess how inclusion of all region scores (1–3) for region 1 (dorsal fin) influenced lesion percentages for that region. Following ANOVA, Tukey's HSD test (Keselman and Rogan 1977) was applied to identify significant pairwise differences in lesion percentages for region 1 across score combinations. ANOVA and Tukey HSD tests were performed for all four regions (dorsal fin, anterior, mid-flank, and peduncle) for both the percentage of lesions and the numeric, observer-averaged scar index.

Regional scores with nonsignificant differences in lesion percentages were grouped, and the percentage of VSA with lesions per whale was calculated as:

As skin abnormality presence was initially assessed per individual rather than per region or image, all confirmed or possible abnormality types were tallied to determine the total number of skin abnormalities detected per whale.

Unlike lesion percentage, which was based on a tally of measured pixels, the scar index was evaluated by examining the percentage of scarring within each region (Robeck et al. 2019). Healing rates of scarring across body regions are known to vary in killer whales, with the dorsal region in particular exhibiting faster recovery (van Weelden et al. 2025). Consequently, averaging scarring rates across all body regions could underestimate an individual's overall scar index. Therefore, regional scores with nonsignificant differences in scar index were aggregated, and a mean scar index for the VSA of each region was calculated per individual. To reduce the number of variables, mean scar indexes for the four regions were combined using principal component analysis (PCA; Maćkiewicz and Ratajczak 1993). PC scores capturing the largest variance (50% or greater) were included in the model set.

Linear models were used to investigate the influence of sample collection location, WCT sex, WCT age, total number of skin abnormalities detected, scar index PCA components, and percentage of lesions on the total number of unique bacterial ESVs and total number of unique bacterial genera detected in flukeprints after normalization. All numeric variables were tested for

collinearity using the *ggpairs* function in the *GGally* package (Schloerke et al. 2024) and for normality using both visual methods (*ggdensity* and *ggqqplot* functions) and the Shapiro–Wilk test (Shapiro and Wilk 1965). Models with the lowest AIC and the greatest adjusted R-squared values were selected, and factors in the final models were releveled using the *relevel* function to conduct pairwise comparisons between factor levels.

Results

A total of 39 eDNA samples were collected in this study, consisting of 24 flukeprints from WCT (11 from California and 13 from B.C.) and 15 seawater eDNA controls (Suppl. material 1: table S2). For nine encounters with WCT, only one flukeprint was collected, resulting in a 1:1 flukeprint-to-seawater control ratio. For the remaining nine encounters, up to three flukeprint samples shared the same seawater control (Suppl. material 1: table S3). After library preparation, a total of 38 samples (21 flukeprints, 14 seawater samples, two extraction negative controls, and one positive control) were sequenced for 16S rDNA. Four samples (three flukeprints and one seawater control) were excluded from sequencing due to insufficient libraries. The total number of reads returned was 15,292,276 (mean = 402,428 reads per sample; Suppl. material 1: table S2). Read counts in both extraction blanks were approximately 30 × lower than the average number of reads per field sample. Samples OB02 and OB17 were excluded from further analyses because matched flukeprint samples did not yield sufficient libraries for sequencing, and samples OB03 and OB04 were excluded because the matched seawater controls did not yield sufficient libraries for sequencing (Suppl. material 1: table S2). One additional sample (OB36) was removed from the dataset prior to rarefaction because it returned fewer than 1,000 reads, which was lower than the number of reads in extraction controls. A total of 233 exact sequence variants (ESVs) were removed from the dataset after filtering for ESVs present in the two extraction negative controls. Rarefaction curves indicated that sequencing depth was sufficient to capture ESV diversity for both seawater controls and flukeprint samples (Suppl. material 1: fig. S4). After the dataset was rarefied and normalized to the 15th percentile of library sizes, 274,749 reads across 2,189 ESVs were retained for further analyses, corresponding to per-sample sequencing depths ranging from 274 to 9,892 reads.

Detection of bacterial taxa

The most abundant bacterial phyla among the top ten detected, based on proportions of ESVs and recovered reads, included Proteobacteria (42% of ESVs and 31% of reads), Bacteroidetes (20% of ESVs and 22% of reads), and Cyanobacteria (9% of ESVs and 23% of reads; Suppl. material 1: fig. S5). Across sample types, seawater control samples produced a total of 1,514 ESVs representing 359 bacterial genera, whereas flukeprint samples produced 1,870 ESVs representing 417 bacterial genera. Overall bacterial ESV richness was highest in B.C. for both seawater controls and flukeprint samples, with flukeprint samples in both locations exhibiting higher mean bacterial ESV richness (Fig. 2).

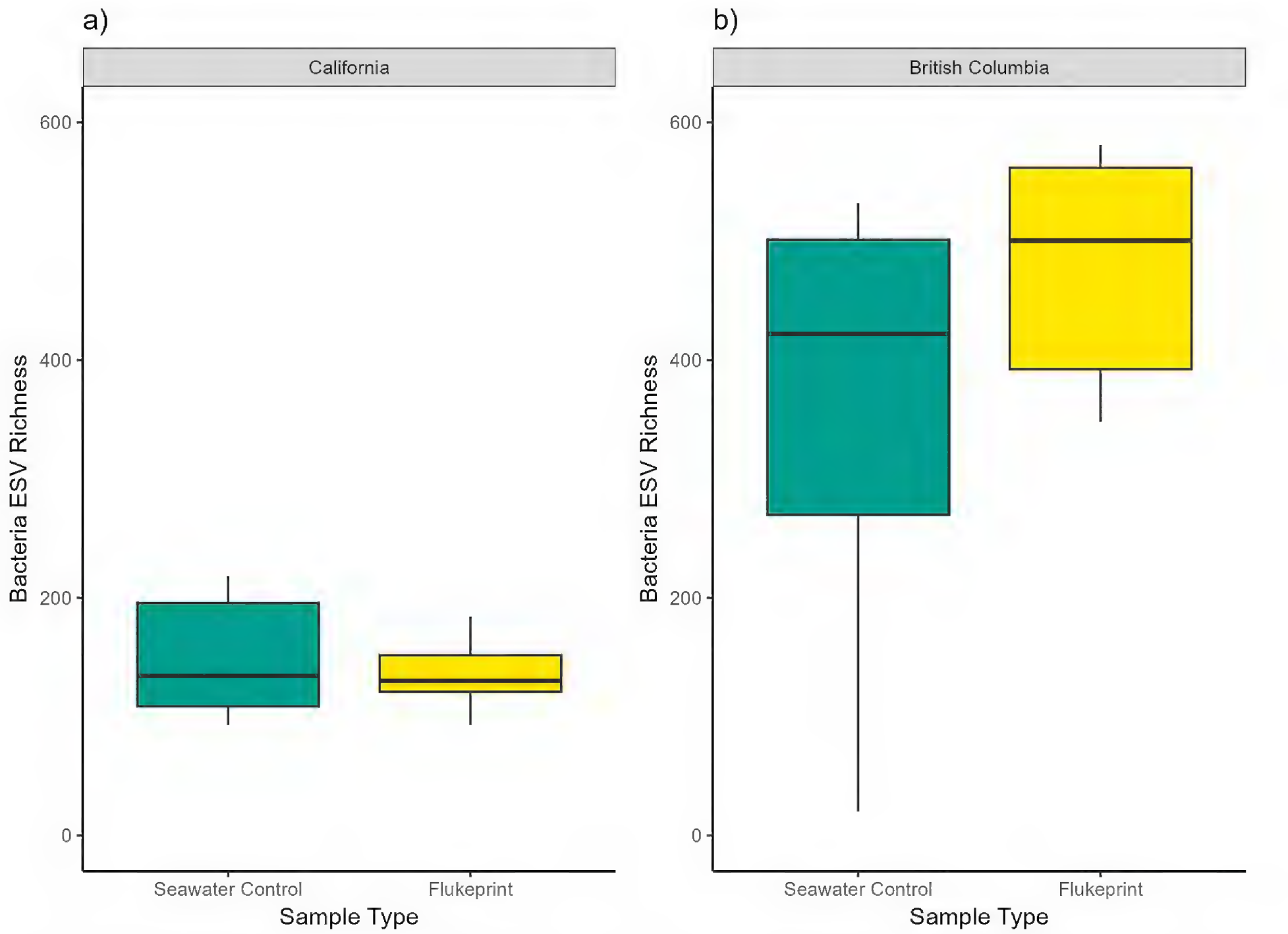

Figure 2. Bacterial exact sequence variant (ESV) richness was higher in samples collected from British Columbia. Bacterial ESV richness varied between seawater controls (green) and flukeprint samples (yellow), as well as between geographic locations (California and British Columbia). Results are based on normalized presence–absence data.

Comparison of bacterial taxa in seawater controls and flukeprints

PCoA of Bray–Curtis dissimilarities revealed some separation between seawater controls and flukeprint samples along the first two axes, which together explained 86.8% of the total variation (Fig. 3); however, these differences were not statistically significant ($R^2 = 0.035$, $p = 0.360$).

A total of 11 flukeprint samples contained bacterial classes that were not detected in seawater controls, with *Acidiobacteria_Gp3* being the most common (Suppl. material 1: fig. S6). Some flukeprints contained more than one unique bacterial class (i.e., OB23, OB32, OB37, and OB38), with three unique classes detected in OB38 (*Acidobacteria_Gp7*, *BCR1_genus_incertae_sedis*, and *WPS-2_genera_incertae_sedis*; Suppl. material 1: fig. S6). Assessment of bacterial orders and genera indicated a greater diversity of ESVs in flukeprint samples, even when the same higher-level taxa were also detected in seawater controls (Suppl. material 1: fig. S7). In total, 300 bacterial genera were shared between seawater controls and flukeprint samples, with 51 genera unique to seawater controls and 117 genera unique to flukeprint samples (Suppl. material 1: fig. S8).

Using a paired approach, flukeprints and their corresponding seawater controls per whale encounter shared an average of 52 (±48) bacterial genera per comparison (Suppl. material 1: table S3), while seawater controls and

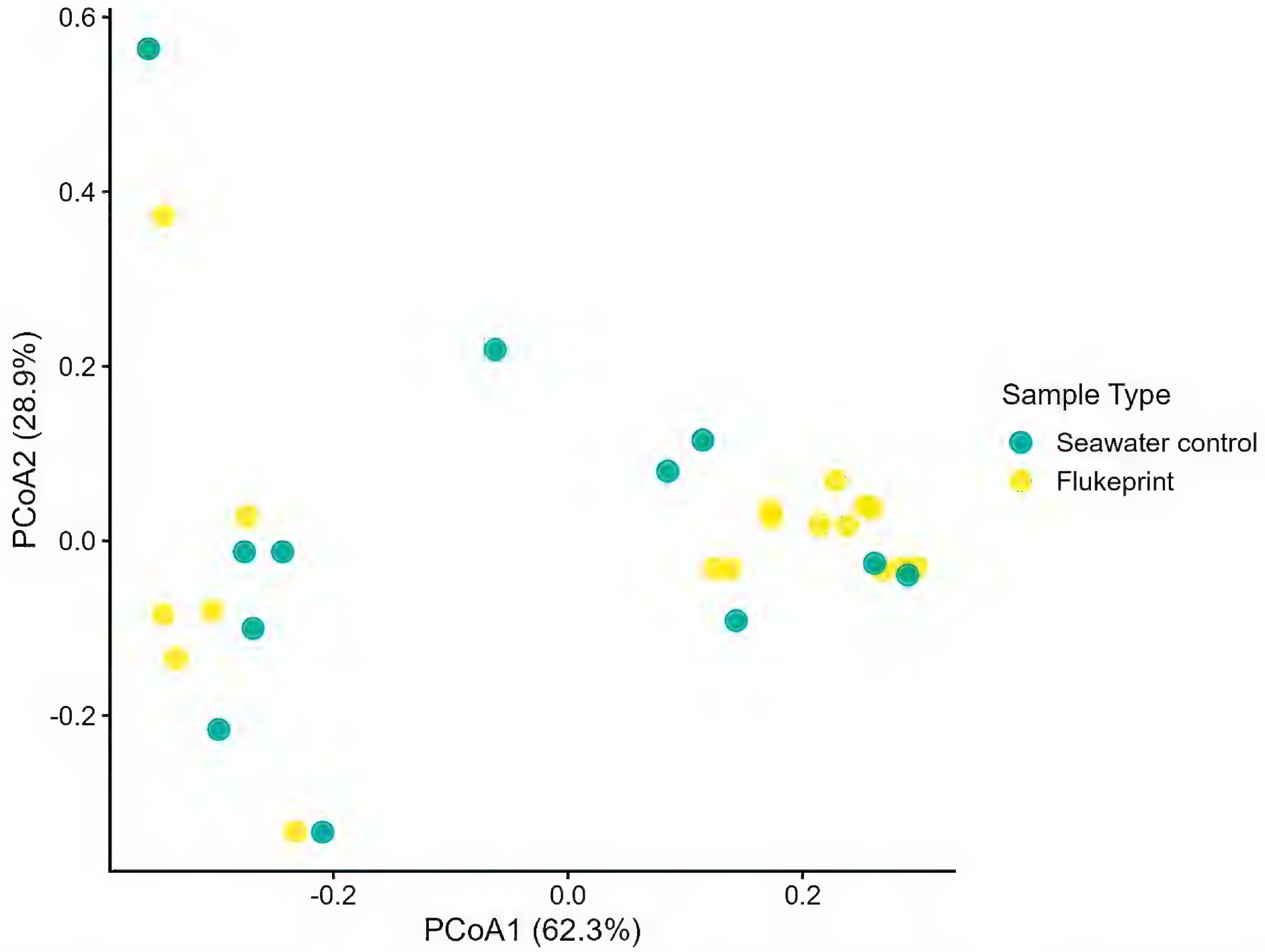

Figure 3. Sample type explains 86.8% of the variation in microbial community composition. Principal coordinates analysis (PCoA) of Bray–Curtis dissimilarities among exact sequence variants (ESVs) shows some clustering of seawater controls (green) and flukeprint samples (yellow).

flukeprints each contained an average of 28 (±15) unique bacterial genera (Suppl. material 1: table S3). All pairwise comparisons reported bacterial genera unique to flukeprint samples only (range: 8–66; Suppl. material 1: figs S9, S10). Flukeprint samples collected during the same encounter generally shared bacterial genera detected in the seawater control, with between zero and 31 genera shared among flukeprint samples (Suppl. material 1: fig. S10).

Bacterial assemblages differed most strongly between geographic locations, compared with differences between seawater controls and flukeprint samples or between whales of different sexes (Suppl. material 1: fig. S11). Permutational analysis of variance (PERMANOVA) showed that geographic location explained 47% of the variation in assemblage composition ($F = 23.8$, $p < 0.001$; Suppl. material 1: table S4). PERMANOVA also indicated that 14% of the variation was explained by whale age ($F = 1.31$, $p = 0.192$), 10% by whale sex ($F = 0.89$, $p = 0.406$), and only 3% by sample type (i.e., seawater controls versus flukeprint samples; $F = 0.92$, $p = 0.761$; Suppl. material 1: table S4). For ESVs unique to flukeprint samples, bacterial assemblages varied between geographic locations and between whales of different sexes (Fig. 4). PERMANOVA results indicated that geographic location explained 9% of the variation in assemblage composition ($F = 1.67$, $p < 0.001$; Suppl. material 1: table S5), whale sex explained 13% ($F = 1.1$, $p = 0.095$), and whale age class explained 12% ($F = 1.01$, $p = 0.381$; Suppl. material 1: table S5).

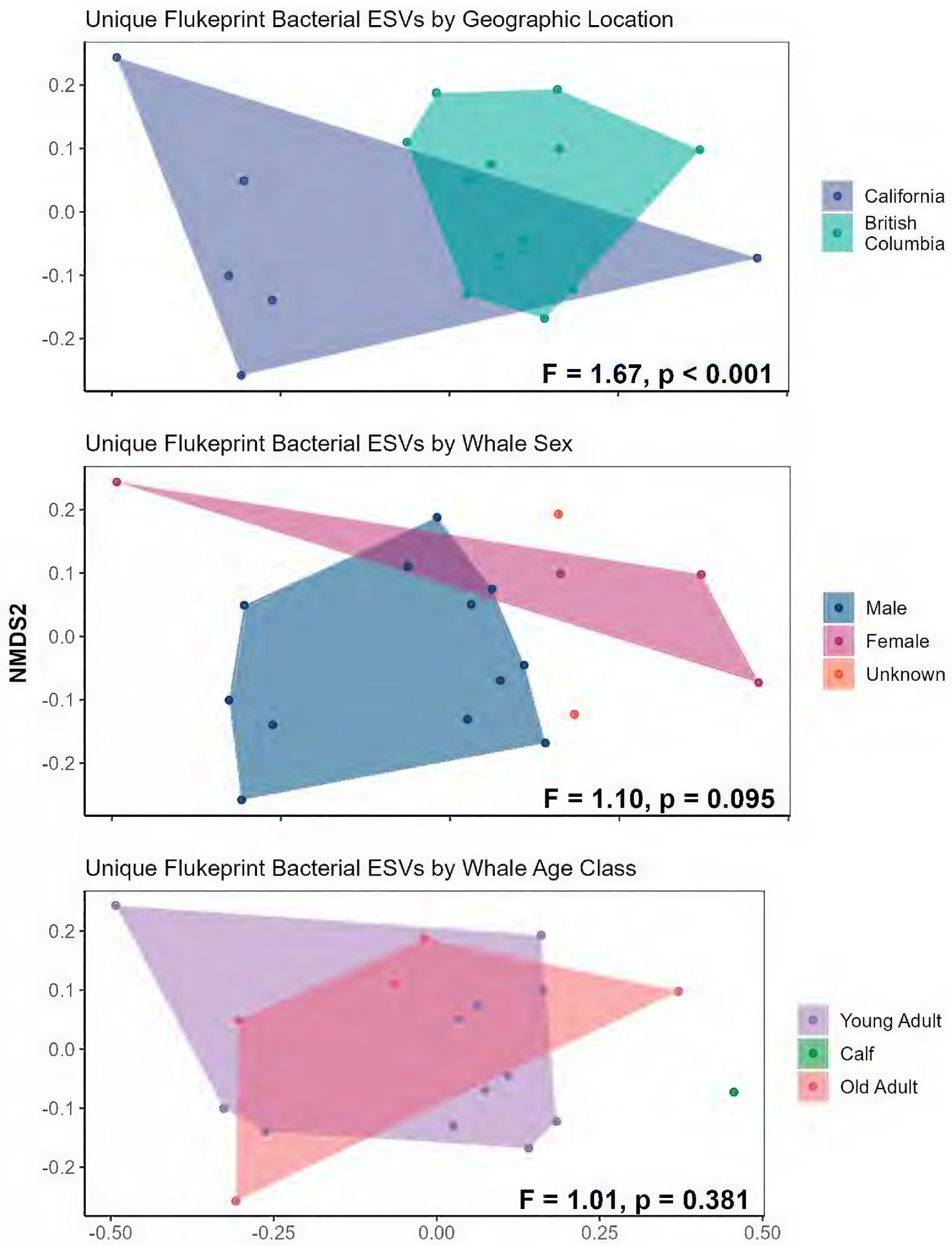

Figure 4. Variation in unique bacterial exact sequence variants (ESVs) is driven primarily by geographic location. Non-metric multidimensional scaling (NMDS) plots of unique bacterial ESVs detected in flukeprint samples display Bray–Curtis dissimilarities between geographic locations (top plot), overlap between whale sexes (center plot), and overlap between whale age classes (bottom plot; stress = 0, R^2 = 0.98). The same ordination is shown in each panel. Ordination is based on normalized abundance data.

Table 1. Top 10 bacterial genera found exclusively in environmental DNA (eDNA) flukeprint samples and previously reported isolations of the taxonomic groups. Taxa are ranked by number of exact sequence variants (ESVs) detected (3–6 ESVs) and relevance to cetacean or marine mammal skin microbiomes (all ESVs scoring 2). Results are based on normalized presence–absence data.

ESVs	Family	Genus	Previous isolation
6	Flavobacteriaceae	*Chryseobacterium*	Marine mammal skin microbiomes (Bai et al. 2021)
6	Thiotrichaceae	*Leucothrix*	Temperate marine bacteria associated with seaweeds (Kelly and Brock 1969; Bland and Brock 1973)
5	Shewanellaceae	*Shewanella*	Killer whale and bowhead whale skin (Dominguez-Sanchez et al. 2023)
			Bryde's whale skin abscess (Li et al. 2020)
3	Prevotellaceae	*Prevotellamassilia*	Human gut (Ndongo et al. 2016)
3	Azonexaceae	*Dechloromonas*	Aquatic environments (Zhang et al. 2021)
2	Carnobacteriaceae	*Carnobacterium*	Baleen whale faecal microbiome (Glaeser et al. 2022)
			Killer whale and bowhead whale skin (Dominguez-Sanchez et al. 2023)
2	Pseudoalteromonadaceae	*Pseudoalteromonas*	Humpback whale skin microbiome (Bierlich et al. 2018)
			Captive killer whale skin microbiome (Chiarello et al. 2017)
2	Granulosicoccaceae	*Granulosicoccus*	Coastal marine environments (Kang et al. 2018)
2	Marinilabiliaceae	*Saccharicrinis*	Marine environments (Yang et al. 2014)
2	Oscillospiraceae	*Butyricicoccus*	Beluga gut microbiomes (Bai et al. 2021)

From evaluation of bacterial genera unique to sample types, a total of 158 ESVs (8%) were found exclusively in flukeprints, and 57 ESVs (4%) were found only in seawater controls. Among the distinct bacterial genera identified in flukeprint samples, the most abundant were *Chryseobacterium* and *Leucothrix* (6 ESVs each), followed by genera including *Shewanella* (5 ESVs) and *Carnobacterium* (2 ESVs; Table 1). The most dominant bacterial genera shared between seawater controls and flukeprints included *Candidatus Pelagibacter* (203 and 240 ESVs; 13% and 14%, respectively), *Bacillariophyta* (126 and 123 ESVs; 8% and 7%, respectively), and *Flavobacterium* (84 and 97 ESVs; 5% and 6%, respectively; Suppl. material 1: table S6). Less abundant shared genera included *Acinetobacter* (11 and 9 ESVs; 0.6% and 0.5%, respectively) and *Novosphingobium* (9 and 14 ESVs; 0.5% and 1%, respectively; Suppl. material 1: table S6).

Individual whale bacterial communities

Out of all sampled whales analyzed, a total of 16 flukeprints (89%) detected unique bacterial families (Fig. 5). All bacterial families detected in whales from California were unique to that location, including *Pasteurellaceae*, *Sphaerochaetaceae*, and *Pseudonocardiaceae*. In comparison, several unique bacterial families were shared among multiple whales from B.C., including *Dysgonomonadaceae* (five individuals), *Marinilabiliaceae* (four individuals), and *Granulosicoccaceae* (four individuals; Fig. 5). The whale with the highest diversity of bacterial families detected was T101A, a mature male WCT (Fig. 5).

FEAST source-tracking analysis showed that flukeprints contained contributions from both known seawater sources and unknown sources in each region (Fig. 6). For whales sampled in California, the proportion of known contributions from seawater per flukeprint ranged from 59–90%, while unknown sources, likely originating from the sampled individual whale, accounted for 20–41% of the total (Table 2; Fig. 6). For samples collected in B.C., known seawater source contributions ranged from 94–100%, whereas unknown sources accounted for

Figure 5. Unique bacterial families detected varied among individual whales. Bacterial exact sequence variant (ESV) richness for unique families detected in flukeprints collected from West Coast Transient killer whales (*Orcinus orca*), faceted by geographic location. Only individual samples that detected unique bacterial families are plotted. White cells indicate zero ESVs. Only ESVs taxonomically assigned to families with high confidence (bootstrap support ≥ 0.80) are included. Results are based on normalized abundance data.

0–6% of the total (Table 2; Fig. 6). This analysis is exploratory in nature, and results should be interpreted cautiously because the study focuses on low-biomass samples with a strong and heterogeneous seawater component.

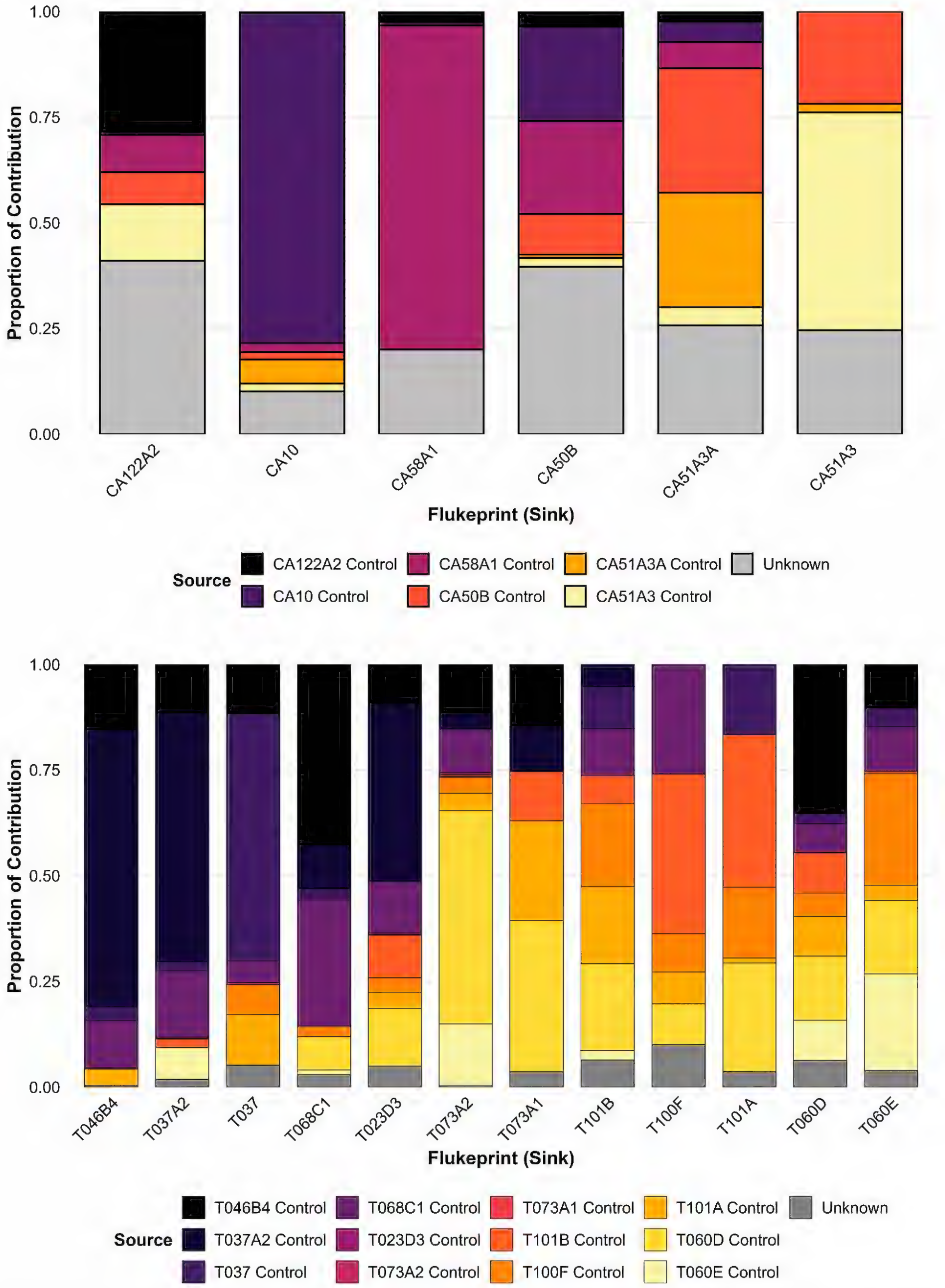

Figure 6. FEAST analysis indicates that flukeprints were largely representative of their corresponding seawater controls. Proportions of known and unknown (gray) bacterial genera detected in flukeprints, analyzed within each geographic location (California and British Columbia). Results are based on normalized abundance data.

Table 2. Known seawater sources contributed a greater proportion of bacteria to flukeprints than unknown sources. Contributions of seawater controls and unknown sources to each flukeprint (sink) sampled in California and British Columbia. Results are based on normalized abundance data.

Flukeprint (Sink)	Bacteria from known seawater sources	Bacteria from unknown source
CA122A2	0.59	0.41
CA10	0.90	0.40
CA58A1	0.80	0.20
CA50B	0.60	0.40
CA51A3A	0.74	0.26
CA51A3	0.75	0.25
T046B4*	1.00	0.00
T037A2*	0.98	0.02
T037	0.95	0.05
T068C1	0.97	0.03
T023D3⁺	0.95	0.05
T073A2⁺	1.00	0.00
T073A1⁺	0.96	0.04
T101B	0.94	0.04
T100F^	0.90	0.10
T101A^	0.96	0.04
T060D~	0.94	0.06
T060E~	0.96	0.04

* Shared seawater control (OB24); ⁺ Shared seawater control (OB34); ^ Shared seawater control (OB39); ~ Shared seawater control (OB42).

Using PICRUSt2, a total of 3,729 enzymes and 520 metabolic pathways were inferred from bacterial ESVs unique to flukeprints. The top 20 most abundant enzymes ranged in abundance from 4,699 to 16,981 reads, and the top 20 pathways ranged from 369,746 to 979,097 reads (Suppl. material 1: tables S7–S8, fig. S12). Oxidoreductases (e.g., thioredoxin-dependent peroxiredoxin), hydrolases (e.g., serine-type D-Ala-D-Ala carboxypeptidase), and transferases (e.g., histidine kinase and non-specific serine/threonine protein kinase) were identified as likely skin-associated enzymes, whereas the remaining enzymes were more commonly associated with general seawater environments (Lebeer et al. 2008; Grice and Segre 2011; Chiarello et al. 2017; Callewaert et al. 2020).

For metabolic pathways, 14 of the top 20 were classified as likely skin-associated (Suppl. material 1: table S8). The relative predicted abundance of both skin-associated and general pathways varied among individual whales (Fig. 7). A subset of whales (i.e., OB05, OB08, OB11, OB18, and OB21) exhibited lower predicted pathway abundances compared with other sampled individuals (Fig. 7), whereas whales T101A and T101B showed the highest predicted abundances across all pathways (Fig. 7). Skin-associated pathways predicted by PICRUSt2 were significantly more abundant than general pathways across all whales sampled (paired t-test, $t = -10.919$, df = 17, $p < 0.00002$, mean difference ≈ 2,862,143). Similar to the FEAST results, these PICRUSt2 outputs should be interpreted with caution. Predicted functional profiles derived from PICRUSt2 represent phylogenetically inferred functional potential rather than empirically

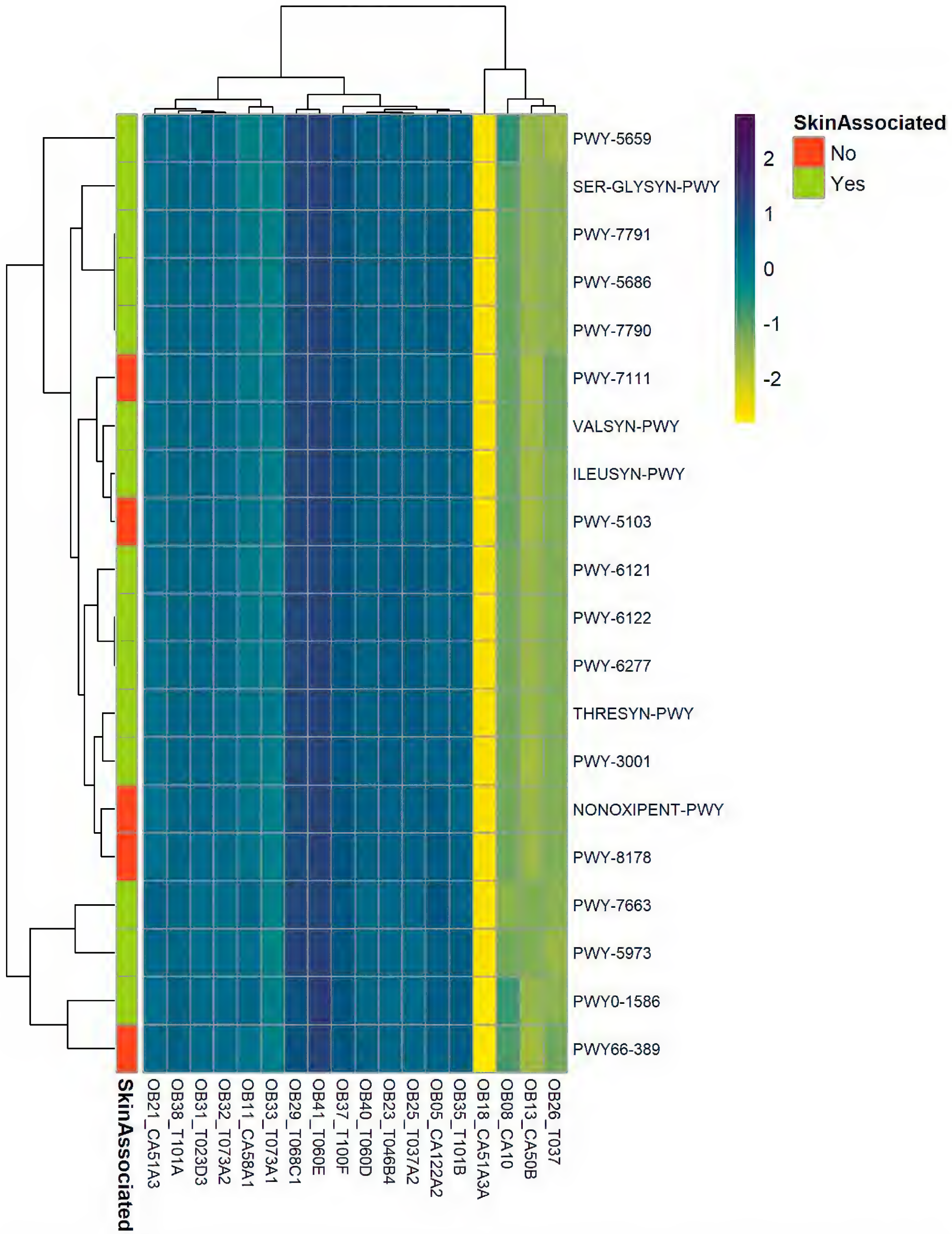

Figure 7. Most predicted pathways from exploratory analyses suggest some degree of skin association, with whales sampled in California exhibiting lower overall predicted abundances across pathways. Predicted abundances for the top 20 pathways identified from unique bacterial exact sequence variants (ESVs) detected in flukeprints. Results are based on normalized abundance data.

measured pathways and are particularly uncertain in diverse, low-biomass marine eDNA samples (Douglas et al. 2020; Sun et al. 2020). These results are therefore reported as exploratory patterns rather than mechanistic evidence.

Image evaluation and scoring

A single left and right lateral image for a total of 15 WCT with unique flukeprint bacteria (four sampled in California and 11 in B.C.) were assessed for skin integrity (Suppl. material 1: table S9). The mean number of days between flukeprint and image collection was 21.5 (range = 0–116; mode = 0). Results of analysis of variance (ANOVA) and Tukey HSD (honestly significant difference) tests showed that the effect of region score on the regional scarring index calculation was negligible ($p > 0.05$), permitting inclusion of all regional measurements. For lesion percentage, there was no significant difference when including all three region scores for the anterior, mid-flank, and peduncle body regions. However, inclusion of dorsal fin regions scored 1 (low quality) significantly affected the mean ($F = 4.616$, $p = 0.019$) and was therefore excluded from further analyses.

Skin lesions and scarring

Gray patches were the most common lesion observed (Suppl. material 1: table S10). Cephalopod lesions were the only lesion type present in WCT sampled in California but not detected in WCT sampled in B.C. CA51A3A exhibited the greatest number of skin abnormalities (six types), whereas T060D and T101A exhibited the fewest (zero types; Suppl. material 1: table S9). During the image-sorting process, it was evident that T060D exhibited a wound posterior to the blowhole that was not visible in the standardized lateral images used to assess skin integrity but was included in the summary of skin abnormalities detected (Suppl. material 1: table S10).

Regional differences in lesion coverage and scar intensity of the visible surface area (VSA) were observed across all individuals (Fig. 8). The mid-flank and peduncle regions displayed more pronounced lesions and scarring compared with the dorsal fin and anterior regions. CA51A3A and T046B4 presented with the greatest severity of total visible lesions (Fig. 8). All sampled WCT demonstrated a high degree of scarring across the measured VSA, with the extent exceeding 75% for CA10, T023D3, and T046B4 (Fig. 8).

The mean percentage of lesions was marginally higher in WCT sampled in California compared with WCT sampled in British Columbia but was not statistically significant ($p = 0.056$; Suppl. material 1: fig. S13). The influence of sex on lesion percentage was also not significant ($p = 0.076$), with lesion coverage only slightly elevated in females compared with males.

Skin integrity analysis

PC1 and PC2 explained 78.2% of the total variance in the regional scar index. PC1 encompassed the amount of scarring across all four regions, explaining 47.5% of the total variance, while PC2 explained differences in scarring distribution and accounted for 30.7% of the total variance.

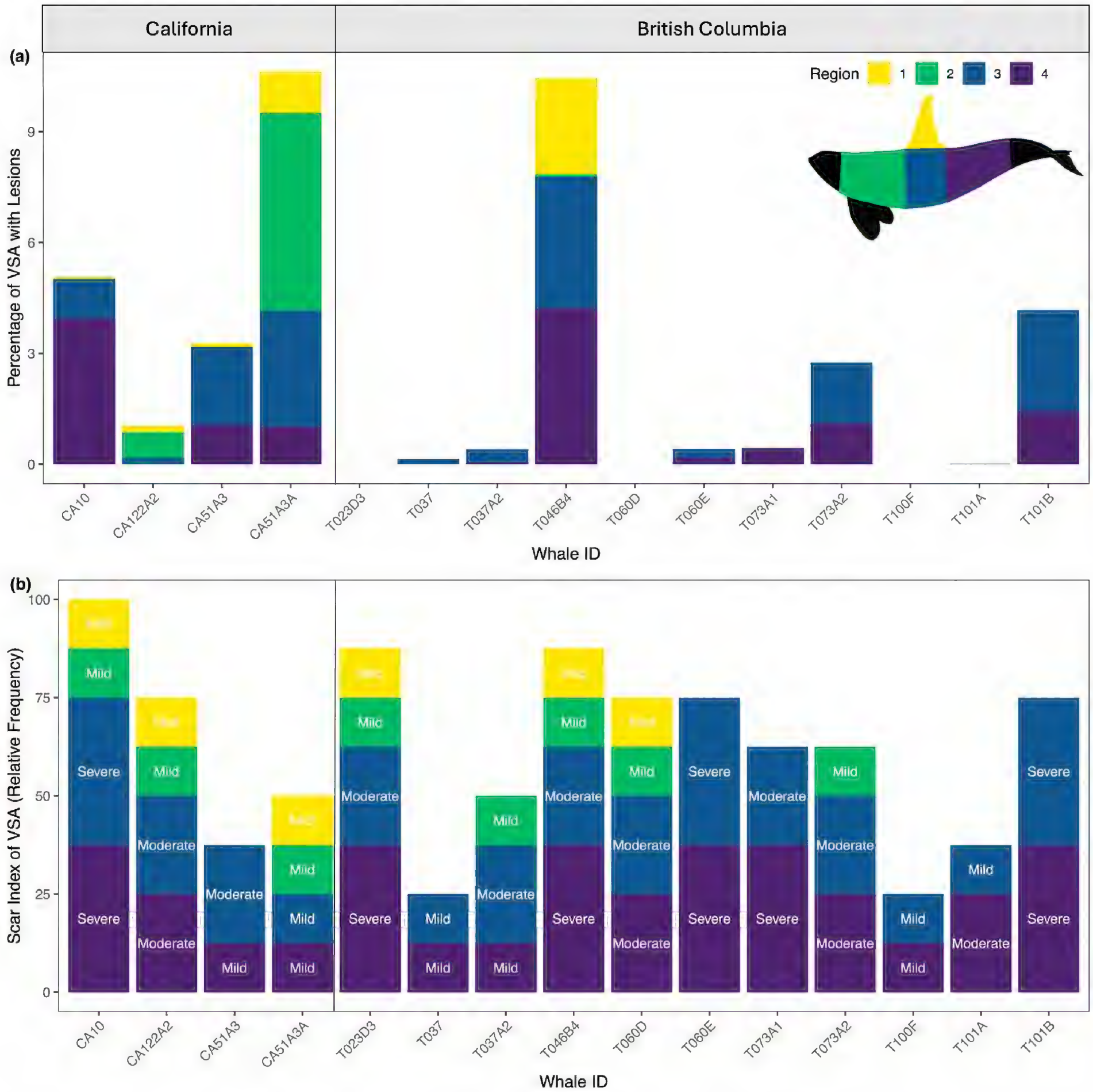

Figure 8. Extent of scarring and lesions varied among individual whales, with higher proportions of both scars and lesions observed in whales sampled in California. Comparisons of (**a**) percentage of lesions and (**b**) scar index recorded for the visible surface area (VSA) of four body regions (1: dorsal fin, 2: anterior, 3: mid-flank, 4: peduncle) of individual West Coast Transient killer whales (*Orcinus orca rectipinnus*) sampled in California and British Columbia with unique flukeprint bacteria. A regional scar index equal to "None" is not shown.

We found that the number of lesion types was correlated with the percentage of VSA with lesions; as a result, only lesion percentage was included in the models. The dataset was not normally distributed, resulting in both total ESVs and total bacterial genera being log-transformed to meet normality assumptions. The final linear model for total ESVs explained 91.7% of the variance and included the following variables: age, month, and percentage of VSA with lesions (AIC = 12.56; Suppl. material 1: table S11, fig. S14). Significant variables included age ($t = -2.821$, $p < 0.05$) and comparisons of total unique ESVs detected between specific months: January with April, February, and

October; February with all other months; April with all other months; October with January, February, and November; and November with February, April, and October (Suppl. material 1: table S12). The final linear model for total bacterial genera explained 96.5% of the variance and included the following variables: region, sex, and month (AIC = 120.6; Suppl. material 1: table S12, fig. S15). Significant variables included comparisons of total unique bacterial genera detected between specific months: January with April, February, and October; February with January, April, and November; April with January, February, and October; October with January, February, and April; and November with January, February, and April. Region (California) was significant for all month levels except January (Suppl. material 1: table S13). The total number of unique ESVs was predicted to decrease with age and with increasing percentage of VSA affected by lesions (Suppl. material 1: fig. S14). The total number of unique bacterial genera was predicted to be higher in B.C. and in male whales (Suppl. material 1: fig. S15). Monthly trends were similar for total unique bacterial ESVs and genera, with increases predicted during the first two months of the year and in November (Suppl. material 1: figs S14, S15). For unique ESVs, the lowest values were predicted for October, whereas for unique genera, the lowest values were predicted for April (Suppl. material 1: figs S14, S15).

Due to the small sample size and limited statistical power of these tests, these results are considered exploratory and should be interpreted accordingly.

Discussion

Flukeprint eDNA sampling has previously been shown to be a valuable source of shed DNA for numerous cetacean species (Parsons et al. 2018; Székely et al. 2021; Alter et al. 2022; Robinson et al. 2024). In this study, we employed flukeprint sampling of WCT to detect unique bacterial genera and compare these results with skin integrity analyses for each individual whale. Functional pathways inferred from unique ESVs detected in flukeprints were either associated with general bacterial metabolic processes or with functions linked to life on host skin, further supporting detection of bacteria related to whale skin microbiomes.

Flukeprint samples were more diverse than seawater control samples, both overall and when analyzing matched samples per flukeprint. *Candidatus Pelagibacter*, a globally distributed ocean bacterium (Leadbetter 2003), was by far the most dominant shared bacterial genus between flukeprints and seawater controls in this study. Similarly, this genus represented up to one quarter of seawater control communities in the St. Lawrence Estuary in a study examining beluga whale (*Delphinapterus leucas*) skin microbiomes (Jia et al. 2022). The majority of dominant bacterial families detected in seawater samples have previously been isolated from coastal surface waters (Bayer et al. 2016; Xu et al. 2016; Sun et al. 2019; Robles-Malagamba et al. 2020), whereas dominant bacterial families detected in flukeprints largely comprised taxa associated with cetacean microbiomes (e.g., Carnobacteriaceae, Pseudoalteromonadaceae, and Shewanellaceae) or with general marine environments (Bland and Brock 1973; Bierlich et al. 2018; Glaeser et al. 2022; Dominguez-Sanchez et al. 2023).

Distinct skin microbiota profiles relative to surrounding seawater have been reported for several cetacean species, including harbor porpoises (*Phocoena*

phocoena) and killer whales (Van Cise et al. 2020). A study of captive killer whales similarly reported higher diversity in individual skin microbiomes compared with tank water (Chiarello et al. 2017). Other studies, including two focused on humpback whale (*Megaptera novaeangliae*) skin microbiomes (Apprill et al. 2011b, 2014), likewise found higher diversity in skin samples relative to surrounding seawater. Unlike studies that sample skin directly, our study did not detect a significant difference in bacterial ESV richness between flukeprint and seawater samples. Because seawater served as the sampling medium for microbial collection, a higher proportion of shared bacterial taxa between sample types is expected due to background seawater communities.

Overall, geographic location was the strongest driver of bacterial diversity, regardless of sample type or individual whale. Regional influences on seawater bacterial communities have been widely documented (Zinger et al. 2011; Lindström and Langenheder 2012; Apprill et al. 2014; Hooper et al. 2019; Zorz et al. 2019). Actinobacteria and Bacteroidetes have been reported as dominant bacterial groups within both the Salish Sea (Crummett 2020; Rhodes et al. 2024) and the Monterey Bay region (Ul-Hasan et al. 2019; Reji et al. 2020). One notable difference between regions was the greater representation of the phylum Thaumarchaeota (family Nitrosopumilaceae) in B.C. samples. Although this archaeal family is widespread in marine environments (Qin et al. 2016), previous studies have shown broader and more consistent distributions in Salish Sea waters compared with more seasonal occurrences in Monterey Bay (Mueller et al. 2015; Rhodes et al. 2024).

Consistent with seawater patterns, bacterial signatures in flukeprints also varied by geographic location. Similar geographic structuring has been reported in skin microbiome studies of killer whales (Hooper et al. 2019), beluga whales (Van Cise et al. 2020), and humpback whales (Bierlich et al. 2018). Several bacterial genera detected uniquely in flukeprints correspond to taxa previously reported from cetacean skin microbiomes. The most abundant genera included *Chryseobacterium*, previously isolated from marine mammal skin (Bai et al. 2021); *Carnobacterium*, isolated from killer whale and bowhead whale skin (Dominguez-Sanchez et al. 2023) as well as baleen whale fecal samples (Glaeser et al. 2022); and *Shewanella*, reported from killer whale and bowhead whale skin (Dominguez-Sanchez et al. 2023) and from a Bryde's whale skin abscess (Li et al. 2020). Other detected genera included *Pseudoalteromonas*, previously identified in humpback whale and captive killer whale skin microbiomes (Chiarello et al. 2017; Bierlich et al. 2018), and *Butyricicoccus*, reported from beluga gut microbiomes (Bai et al. 2021). Several genera detected exclusively in this study are typically associated with marine environments, including *Leucothrix*, *Dechloromonas*, and *Granulosicoccus* (Kelly and Brock 1969; Kang et al. 2018; Zhang et al. 2021). Members of the family Flavobacteriaceae play key roles in mineralization of organic matter in marine environments (Bowman and Nichols 2005; Sehnal et al. 2021) and may contribute to host skin–environment interactions, particularly under changing water quality conditions (Sehnal et al. 2021). This family includes *Polaribacter* and *Tenacibaculum*, which have been identified as core genera in baleen whale skin microbiomes (Bowman and Nichols 2005; Apprill et al. 2011b, 2014; Bierlich et al. 2018; Sehnal et al. 2021).

In odontocetes, *Tenacibaculum* and *Psychrobacter* (family Moraxellaceae) have previously been isolated from deceased (Dominguez-Sanchez et al. 2023), live (Hooper et al. 2019), and captive killer whales (Chiarello et al. 2017), as

well as bottlenose dolphins (*Tursiops truncatus*; Chiarello et al. 2017; Robles-Malagamba et al. 2020). However, we did not detect either of these genera in flukeprint or seawater control samples. The lack of detection of *Polaribacter* in this study and others on wild and captive killer whales (Chiarello et al. 2017; Hooper et al. 2019; Dominguez-Sanchez et al. 2023) suggests that this genus may not be a cosmopolitan skin taxon across all cetacean skin microbiomes. Conversely, *Pseudomonas* has previously been detected in both Delphinidae and Phocidae skin microbiomes, suggesting that members of this genus are a core component of odontocete skin microbiomes (Apprill et al. 2020). For beluga whales and humpback whales, skin microbiomes have been shown to be relatively conserved among individuals within the same region, with skin bacterial communities differing depending on a combination of metabolic state, presence of injury, and geographic region (Aubin et al. 1990; Apprill et al. 2014; Van Cise et al. 2020; Jia et al. 2022). In this study, we found that both total ESVs and total bacterial genera from WCT flukeprints differed significantly depending on whether whales were sampled in spring, fall, or winter, with sampling month emerging as a significant predictor of bacterial ESV and genus richness. A study on rorqual skin microbiota structure in the eastern South Pacific reported comparable seasonal patterns (Toro et al. 2021). Notably, age exhibited a significant negative relationship with skin bacterial diversity in WCT, with higher flukeprint bacterial ESV diversity observed in younger individuals, a pattern previously reported in blue whales (*Balaenoptera musculus*), fin whales (*Balaenoptera physalus*), and humpback whales (Toro Cortes 2019). The skin microbiome has also been linked to age in humans and birds (Lucas et al. 2005) and may be influenced by sex (Ross et al. 2018), which in this study explained part of the observed variation in bacterial assemblage composition (Leyden et al. 1975; Capone et al. 2011).

In terms of functional predictions for unique flukeprint ESVs, differences were observed in enzyme class representation between skin-associated and general pathways. Oxidoreductases (EC 1), transferases (EC 2), and hydrolases (EC 3) were proportionally higher among pathways likely associated with skin. These enzyme classes have established roles in supporting microbial survival and colonization on skin through oxidative stress protection, adhesion, and degradation of host lipids and proteins (Lebeer et al. 2008; Fitz-Gibbon et al. 2013; Callewaert et al. 2020). In contrast, lyases (EC 4), isomerases (EC 5), and ligases (EC 6) were more evenly distributed and likely represent general microbial metabolic capacity (Zhang et al. 2016). We observed overlap between the functional pathways identified in flukeprints and those reported from isolated killer whale and dolphin skin, particularly pathways related to DNA replication and repair, cellular signaling and processing, and glycan biosynthesis (Chiarello et al. 2017). These functions are thought to be driven by the need for rapid microbial growth under conditions of near-constant sloughing into the ocean environment (Hicks et al. 1985; Apprill et al. 2014; Chiarello et al. 2017). However, it is important to note that PICRUSt assessments are limited to previously annotated genes, meaning that undiscovered functional genes are not considered, and the analysis does not account for potential differences in gene expression (Chiarello et al. 2017). Additionally, as noted in the Results, outputs from PICRUSt should be interpreted with caution. The results presented here do not demonstrate the presence of host-associated bacteria but instead suggest pathways that may have skin-related functions. Finally, because information on the functional relevance of cetacean microbiomes remains limited

(Li et al. 2022), this analysis was restricted to a single study that extended beyond taxonomic identification to functional classification (Chiarello et al. 2017).

In addition to the functional relevance of bacteria detected in flukeprints, it is also important to note the absence of human-associated bacteria in the samples. Previous studies have detected genera such as *Enhydrobacter*, *Paracoccus*, and *Sphingomonas* on the skin of captive killer whales and dolphins, taxa considered absent in wild odontocete skin microbiomes but common on human skin (Apprill et al. 2014, 2020; Perez Perez et al. 2016; Chiarello et al. 2017; Van Cise et al. 2020). These findings were interpreted as evidence of bacterial transfer from caretakers in captive environments (Chiarello et al. 2017) and, in the present study, support the lack of human contamination during sample collection and processing. Similarly, no key killer whale respiratory bacteria (e.g., *Staphylococcus*, *Bacillus*, and *Vibrio*) were detected in flukeprints, despite these bacteria likely being present in exhaled breath that would eventually settle on the sea surface (Raverty et al. 2017; Rhodes et al. 2022). This suggests that the respiratory microbiome does not contribute substantially to the bacterial communities detected in flukeprints.

Similar to differences observed between respiratory and skin microbial communities, bacterial assemblages are also known to vary across different body regions of cetaceans (Chiarello et al. 2017). This variation has been documented in captive cetaceans but is nearly impossible to assess in wild individuals. Existing skin microbiome studies of free-living cetaceans primarily rely on biopsy sampling, which typically yields a 5–8 mm diameter epidermal sample from the upper lateral portion of the body (Mathews et al. 1988; Noren and Mocklin 2012; Van Cise et al. 2020; Eldridge et al. 2022; Jia et al. 2022). For odontocetes, which exhibit higher skin shedding rates than mysticetes (Hicks et al. 1985; Aubin et al. 1990; Hooker et al. 2002; Pitman et al. 2020), flukeprint sampling provides an alternative approach to assess bacteria shed from skin cells across the entire body surface, rather than from the limited area represented by a biopsy.

Consistent with bacterial patterns detected in flukeprints, skin lesion coverage varied among individuals and between geographic locations. Gray patches were the most common lesion type observed, as previously reported in Southern Resident killer whales (Gaydos et al. 2023). WCT sampled in California exhibited higher percentages of skin lesions compared with whales sampled in B.C., suggesting that local environmental conditions in California waters may contribute to lesion development (Mouton and Botha 2012). A previous study of bottlenose dolphins in Monterey Bay found that approximately 90% of individuals exhibited skin lesions (Maldini et al. 2010). More broadly, studies of bottlenose dolphins in Santa Monica Bay reported extensive skin lesions of bacterial origin (Bearzi et al. 2009). Investigations of skin conditions in North Atlantic right whales (*Eubalaena glacialis*) (Hamilton and Marx 2005) and other odontocete species have suggested that factors such as untreated wastewater and chemical pollution contribute to lesion prevalence (Bressem et al. 2007; Kiszka et al. 2009). With respect to lesion coverage and bacterial diversity, studies of humpback whales (Apprill et al. 2011b) and bowhead whales (Dominguez-Sanchez et al. 2023) reported lower bacterial diversity in health-compromised individuals. In the present study, we observed a similar pattern, with a nonsignificant negative relationship between percentage of skin lesions and bacterial ESV diversity in WCT. In odontocetes, a study of Antarctic killer whales reported more variable skin microbiomes in individuals with poor skin condition (Hooper

et al. 2019), whereas beluga whales showed no difference in bacterial diversity between healthy individuals and those with skin disease (Van Cise et al. 2020).

Unlike skin lesions, no effect of scarring intensity on bacterial skin taxa was detected. Many of the scars observed were rake marks, which are common in killer whales and result from social interactions such as aggression and play (Robeck et al. 2019; van Weelden et al. 2025). Although rake-inflicted wounds may introduce infections (Raverty et al. 2020), scarring was present in all individuals sampled. WCT are characterized by particularly extensive scarring (Towers et al. 2019), which likely contributed to the absence of a detectable effect. Comparisons with populations that exhibit less scarring, such as Southern Resident killer whales, may provide further insight into the influence of scarring on killer whale skin microbiomes in future studies.

Whilst flukeprints may provide an opportunity for representative bacterial skin sampling, there are current limitations to this approach. The primary challenge in this study was the lack of existing information on bacterial skin microbiomes and functional pathways of free-living WCT, which made comparisons between detected bacterial taxa and functional pathways and those reported in the literature difficult. Second, skin integrity assessments were limited to the visible surface area in standardized lateral images, potentially missing skin abnormalities present in other body regions. Lighting variability in the dorsal region may have further reduced assessment accuracy, as noted in a previous killer whale skin assessment study (Robeck et al. 2019).

Conclusion

Here, we demonstrate the application of non-invasive flukeprint sampling to obtain information on bacterial taxa likely derived from the skin of WCT. Variability in skin-associated bacteria and skin integrity is likely influenced by a combination of demographic and environmental factors, with a higher prevalence of skin lesions observed in WCT sampled in California. The non-invasive nature of this approach, combined with the greater level of skin surface representation afforded by eDNA, suggests that flukeprint sampling could be applied to other odontocete populations or species to better understand core skin microbiomes. For populations such as the Southern Residents, flukeprint eDNA sampling provides an opportunity to assess health status with limited disturbance compared to biopsy sampling. Understanding differences in functional pathways among individuals with varying skin conditions and identifying the prevalence of potentially pathogenic bacteria may further inform population health risk assessments and support enhanced conservation management measures. Over the longer term, as artificial intelligence algorithms continue to be developed (Murphy et al. 2025), integrating flukeprint eDNA data with lateral and drone imagery may facilitate machine-learning approaches to detect, characterize, and classify skin lesions in wild cetaceans.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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The MetaWorks v1.9.3 multi-marker metabarcode bioinformatic pipeline is available at https://github.com/terrimporter/MetaWorks. The datasets generated and/or analyzed during the current study, along with associated R scripts, are available in the GitHub repository https://github.com/chloerobinson23/OrcaBiome-2025. Raw sequence files are available from the NCBI Sequence Read Archive under BioProject ID PRJNA1280689 (Zenodo DOI: https://doi.org/10.5281/zenodo.17316820)

Author contributions

Conceptualization (CVR and BCVK); Methodology (CVR, BCVK, and MWDJ); Formal analysis (CVR, BCVK, EJL, and GJS); Investigation (CVR, BCVK, EJL, GJS, CMT, and MWDJ); Resources (CMT); Data curation (CVR, BCVK, EJL, GJS, and CMT); Visualization (CVR and BCVK); Writing—original draft (CVR, BCVK, EJL, and CMT); Writing—review and editing (CVR, BCVK, EJL, CMT, GJS, and MWDJ); Supervision (CVR, CMT, and MWDJ); Project administration (BCVK, EJL, and CMT); Funding acquisition (CVR).

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Acevedo-Whitehouse K, Rocha-Gosselin A, Gendron D (2010) A novel non-invasive tool for disease surveillance of free-ranging whales and its relevance to conservation

programs. Animal Conservation 13(2): 217–225. https://doi.org/10.1111/j.1469-1795.2009.00326.x

Alter SE, King CD, Chou E, Chin SC, Rekdahl M, Rosenbaum HC (2022) Using environmental DNA to detect whales and dolphins in the New York Bight. Frontiers in Conservation Science 3: 820377. https://doi.org/10.3389/fcosc.2022.820377

Apprill A, Mooney TA, Lyman E, Stimpert AK, Rappé MS (2011a) Humpback whales harbour a combination of specific and variable skin bacteria. Environmental Microbiology Reports 3(2): 223–232. https://doi.org/10.1111/j.1758-2229.2010.00213.x

Apprill A, Mooney TA, Lyman E, Stimpert AK, Rappé MS (2011b) Humpback whales harbour a combination of specific and variable skin bacteria. Environmental Microbiology Reports 3(2): 223–232. https://doi.org/10.1111/j.1758-2229.2010.00213.x

Apprill A, Robbins J, Eren AM, Pack AA, Reveillaud J, Mattila D, Moore M, Niemeyer M, Moore KMT, Mincer TJ (2014) Humpback whale populations share a core skin bacterial community: Towards a health index for marine mammals? PLoS ONE 9(3): e90785. https://doi.org/10.1371/journal.pone.0090785

Apprill A, Miller CA, Moore MJ, Durban JW, Fearnbach H, Barrett-Lennard LG (2017) Extensive Core Microbiome in Drone-Captured Whale Blow Supports a Framework for Health Monitoring. mSystems 2: 10.1128/msystems.00119-17. https://doi.org/10.1128/msystems.00119-17

Apprill A, Miller CA, Van Cise AM, U'Ren JM, Leslie MS, Weber L, Baird RW, Robbins J, Landry S, Bogomolni A, Waring G (2020) Marine mammal skin microbiotas are influenced by host phylogeny. Royal Society Open Science 7(5): 192046. https://doi.org/10.1098/rsos.192046

Aubin DJS, Smith TG, Geraci JR (1990) Seasonal epidermal molt in beluga whales, *Delphinapterus leucas*. Canadian Journal of Zoology 68(2): 359–367. https://doi.org/10.1139/z90-051

Avila IC, Kaschner K, Dormann CF (2018) Current global risks to marine mammals: Taking stock of the threats. Biological Conservation 221: 44–58. https://doi.org/10.1016/j.biocon.2018.02.021

Bai S, Zhang P, Zhang C, Du J, Du X, Zhu C, Liu J, Xie P, Li S (2021) Comparative study of the gut microbiota among four different marine mammals in an aquarium. Frontiers in Microbiology 12: 769012. https://doi.org/10.3389/fmicb.2021.769012

Baker CS, Steel D, Nieukirk S, Klinck H (2018) Environmental DNA (eDNA) from the wake of the whales: Droplet digital PCR for detection and species identification. Frontiers in Marine Science 5: 133. https://doi.org/10.3389/fmars.2018.00133

Baker CS, Claridge D, Dunn C, Fetherston T, Baker DN, Klinck H, Steel D (2023) Quantification by droplet digital PCR and species identification by metabarcoding of environmental (e)DNA from Blainville's beaked whales, with assisted localization from an acoustic array. PLoS ONE 18(9): e0291187. https://doi.org/10.1371/journal.pone.0291187

Balvočiūtė M, Huson DH (2017) SILVA, RDP, Greengenes, NCBI and OTT — How do these taxonomies compare? BMC Genomics 18(S2): 114. https://doi.org/10.1186/s12864-017-3501-4

Bayer B, Vojvoda J, Offre P, Alves RJE, Elisabeth NH, Garcia JA, Volland J-M, Srivastava A, Schleper C, Herndl GJ (2016) Physiological and genomic characterization of two novel marine thaumarchaeal strains indicates niche differentiation. The ISME Journal 10(5): 1051–1063. https://doi.org/10.1038/ismej.2015.200

Bearzi M, Rapoport S, Chau J, Saylan C (2009) Skin lesions and physical deformities of coastal and offshore common bottlenose dolphins (*Tursiops truncatus*) in San-

ta Monica Bay and adjacent areas, California. Ambio 38(2): 66–71. https://doi.org/10.1579/0044-7447-38.2.66

Beineke A, Siebert U, Wohlsein P, Baumgärtner W (2010) Immunology of whales and dolphins. Veterinary Immunology and Immunopathology 133(2–4): 81–94. https://doi.org/10.1016/j.vetimm.2009.06.019

Bennington SM, Bourke SD, Wilkinson SP, Englebert N, Bond DM, Jeunen G-J, Dawson S, Slooten E, Dillingham PW, Rayment WJ, Alexander A (2024) New insights into the population structure of Hector's dolphin (*Cephalorhynchus hectori*) revealed using environmental DNA. Environmental DNA 6(5): e70024. https://doi.org/10.1002/edn3.70024

Bertulli C, Cecchetti A, Van Bressem M-F, Van Waerebeek K (2012) Skin disorders in common minke whales and white-beaked dolphins off Iceland, a photographic assessment. Journal of Marine Animals & Their Ecology 5: 29–40.

Bierlich KC, Miller C, DeForce E, Friedlaender AS, Johnston DW, Apprill A (2018) Temporal and regional variability in the skin microbiome of humpback whales along the western Antarctic Peninsula. Applied and Environmental Microbiology 84(5): e02574–e17. https://doi.org/10.1128/AEM.02574-17

Black NA, Talty CM, Schulman-Janiger A, Srinivasan M (2023) Mammal Hunting Killer Whales off Monterey, California: A 30-Year Synthesis. In: Srinivasan M, Würsig B (Eds) Social Strategies of Carnivorous Mammalian Predators: Hunting and Surviving as Families. Springer International Publishing, Cham, 283–333. https://doi.org/10.1007/978-3-031-29803-5_8

Bland JA, Brock TD (1973) The marine bacterium *Leucothrix mucor* as an algal epiphyte. Marine Biology 23(4): 283–292. https://doi.org/10.1007/BF00389335

Bossart GD, Cray C, Solorzano JL, Decker SJ, Cornell LH, Altman NH (1996) Cutaneous papillomaviral-like papillomatosis in a killer whale (*Orcinus orca*). Marine Mammal Science 12(2): 274–281. https://doi.org/10.1111/j.1748-7692.1996.tb00576.x

Bowman JP, Nichols DS (2005) Novel members of the family Flavobacteriaceae from Antarctic maritime habitats including Subsaximicrobium wynnwilliamsii gen. nov., sp. nov., Subsaximicrobium saxinquilinus sp. nov., Subsaxibacter broadyi gen. nov., sp. nov., Lacinutrix copepodicola gen. nov., sp. nov., and novel species of the genera Bizionia, Gelidibacter and Gillisia. International Journal of Systematic and Evolutionary Microbiology 55(4): 1471–1486. https://doi.org/10.1099/ijs.0.63527-0

Bressem M-FV, Waerebeek KV, Reyes JC, Félix F, Echegaray M, Siciliano S, Beneditto APD, Flach L, Viddi F, Avila IC, Herrera JC, Tobón IC, Bolaños-Jiménez J, Moreno IB, Ott PH, Sanino GP, Castineira E, Montes D, Crespo E, Flores PAC, Haase B, de Souza SMFM, Laeta M, Fragoso AB (2007) A preliminary overview of skin and skeletal diseases and traumata in small cetaceans from South American waters. The Latin American Journal of Aquatic Mammals 6(1): 7–42. https://doi.org/10.5597/lajam00108

Bressem M-FV, Raga JA, Guardo GD, Jepson PD, Duignan PJ, Siebert U, Barrett T, Santos MC de O, Moreno IB, Siciliano S, Aguilar A, Waerebeek KV (2009a) Emerging infectious diseases in cetaceans worldwide and the possible role of environmental stressors. Diseases of Aquatic Organisms 86: 143–157. https://doi.org/10.3354/dao02101

Bressem M-FV, Waerebeek KV, Aznar FJ, Raga JA, Jepson PD, Duignan P, Deaville R, Flach L, Viddi F, Baker JR, Beneditto APD, Echegaray M, Genov T, Reyes J, Felix F, Gaspar R, Ramos R, Peddemors V, Sanino GP, Siebert U (2009b) Epidemiological pattern of tattoo skin disease: A potential general health indicator for cetaceans. Diseases of Aquatic Organisms 85: 225–237. https://doi.org/10.3354/dao02080

California Killer Whale Project (2023) California Killer Whale Project 2023 Field Guide, 52 pp. https://www.californiakillerwhaleproject.org/_files/ugd/6354fd_4617a36f2eb74cda9ed126e45570aae8.pdf

Callewaert C, Ravard Helffer K, Lebaron P (2020) Skin microbiome and its interplay with the environment. American Journal of Clinical Dermatology 21(S1): 4–11. https://doi.org/10.1007/s40257-020-00551-x

Capone KA, Dowd SE, Stamatas GN, Nikolovski J (2011) Diversity of the human skin microbiome early in life. The Journal of Investigative Dermatology 131(10): 2026–2032. https://doi.org/10.1038/jid.2011.168

Caspi R, Billington R, Fulcher CA, Keseler IM, Kothari A, Krummenacker M, Latendresse M, Midford PE, Ong Q, Ong WK, Paley S, Subhraveti P, Karp PD (2018) The MetaCyc database of metabolic pathways and enzymes. Nucleic Acids Research 46(D1): D633–D639. https://doi.org/10.1093/nar/gkx935

Centelleghe C, Carraro L, Gonzalvo J, Rosso M, Esposti E, Gili C, Bonato M, Pedrotti D, Cardazzo B, Povinelli M, Mazzariol S (2020) The use of Unmanned Aerial Vehicles (UAVs) to sample the blow microbiome of small cetaceans. PLoS ONE 15(7): e0235537. https://doi.org/10.1371/journal.pone.0235537

Chen H (2022) VennDiagram: Generate High-Resolution Venn and Euler Plots. https://cran.r-project.org/web/packages/VennDiagram/index.html [March 19, 2025]

Chiarello M, Villéger S, Bouvier C, Auguet JC, Bouvier T (2017) Captive bottlenose dolphins and killer whales harbor a species-specific skin microbiota that varies among individuals. Scientific Reports 7(1): 15269. https://doi.org/10.1038/s41598-017-15220-z

Clegg ILK, Butterworth A (2017) Assessing the Welfare of Cetacea. In: Butterworth A (Ed.) Marine Mammal Welfare: Human Induced Change in the Marine Environment and its Impacts on Marine Mammal Welfare. Springer International Publishing, Cham, 183–211. https://doi.org/10.1007/978-3-319-46994-2_12

Cloern JE, Abreu PC, Carstensen J, Chauvaud L, Elmgren R, Grall J, Greening H, Johansson JOR, Kahru M, Sherwood ET, Xu J, Yin K (2016) Human activities and climate variability drive fast-paced change across the world's estuarine–coastal ecosystems. Global Change Biology 22(2): 513–529. https://doi.org/10.1111/gcb.13059

Comeau AM, Li WKW, Tremblay J-É, Carmack EC, Lovejoy C (2011) Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. PLoS ONE 6(11): e27492. https://doi.org/10.1371/journal.pone.0027492

Comeau AM, Douglas GM, Langille MGI (2017) Microbiome Helper: A custom and streamlined workflow for microbiome research. mSystems 2: 10.1128/msystems.00127-16. https://doi.org/10.1128/msystems.00127-16

Corsi E, Calambokidis J, Flynn KR, Steiger GH (2022) Killer whale predatory scarring on mysticetes: A comparison of rake marks among blue, humpback, and gray whales in the eastern North Pacific. Marine Mammal Science 38(1): 223–234. https://doi.org/10.1111/mms.12863

Crain CM, Halpern BS, Beck MW, Kappel CV (2009) Understanding and managing human threats to the coastal marine environment. Annals of the New York Academy of Sciences 1162(1): 39–62. https://doi.org/10.1111/j.1749-6632.2009.04496.x

Crummett LT (2020) Acidification decreases microbial community diversity in the Salish Sea, a region with naturally high pCO2. PLoS ONE 15(10): e0241183. https://doi.org/10.1371/journal.pone.0241183

Dan ME, Portner EJ, Bowman JS, Semmens BX, Owens SM, Greenwald SM, Choy CA (2024) Using low volume eDNA methods to sample pelagic marine animal assemblages. PLoS ONE 19(5): e0303263. https://doi.org/10.1371/journal.pone.0303263

de Bruyn PJN, Tosh CA, Terauds A (2013) Killer whale ecotypes: Is there a global model? Biological Reviews of the Cambridge Philosophical Society 88(1): 62–80. https://doi.org/10.1111/j.1469-185X.2012.00239.x

Dominguez-Sanchez CA, Ferguson SH, Edkins T, Young BG, Kringorn J (2023) Pilot study: Decoding the skin microbiome of bowhead (*Balaena mysticetus*) and killer whales (*Orcinus orca*) in Nunavut, Canada. Arctic Science 10(1): 169–188. https://doi.org/10.1139/as-2023-0028

dos Santos RA, Blabolil P (2025) Comparison of bioinformatic pipelines for eDNA metabarcoding data analysis of fish populations. Fishes 10(5): 214. https://doi.org/10.3390/fishes10050214

Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI (2020) PICRUSt2 for prediction of metagenome functions. Nature Biotechnology 38(6): 685–688. https://doi.org/10.1038/s41587-020-0548-6

Dully V, Wilding TA, Mühlhaus T, Stoeck T (2021) Identifying the minimum amplicon sequence depth to adequately predict classes in eDNA-based marine biomonitoring using supervised machine learning. Computational and Structural Biotechnology Journal 19: 2256–2268. https://doi.org/10.1016/j.csbj.2021.04.005

Dwyer S, Visser I (2011) Cookie cutter shark (*Isistius sp.*) bites on cetaceans, with particular reference to killer whales (Orca) (*Orcinus orca*). Aquatic Mammals 37(2): 111–138. https://doi.org/10.1578/AM.37.2.2011.111

Eldridge SA, Mortazavi F, Rice FL, Ketten DR, Wiley DN, Lyman E, Reidenberg JS, Hanke FD, DeVreese S, Strobel SM, Rosene DL (2022) Specializations of somatosensory innervation in the skin of humpback whales (*Megaptera novaeangliae*). The Anatomical Record : Advances in Integrative Anatomy and Evolutionary Biology 305(3): 514–534. https://doi.org/10.1002/ar.24856

Farrell MJ, Govender D, Hajibabaei M, van der Bank M, Davies TJ (2018). Bacterial diversity in the waterholes of the Kruger National Park: an eDNA metabarcoding approach. Trends in DNA Barcoding and Metabarcoding 1: 229–242. https://doi.org/10.1139/gen-2018-0064

Fisheries and Oceans Canada (2013) Information in Support of the Identification of Critical Habitat for Transient Killer Whales (*Orcinus orca*) off the West Coast of Canada, 14 pp. https://waves-vagues.dfo-mpo.gc.ca/Library/349619.pdf

Fisheries and Oceans Canada (2019) Stock Assessment of Pacific Harbour Seals (*Phoca vitulina richardsi*) in Canada in 2019, 10 pp. https://waves-vagues.dfo-mpo.gc.ca/library-bibliotheque/41073654.pdf

Fisheries and Oceans Canada (2024) 2014 population assessment of Pacific harbour seal (*Phoca vitulina richardsi*) in the Strait of Georgia, 8 pp. https://publications.gc.ca/collections/collection_2024/mpo-dfo/fs70-6/Fs70-6-2024-027-eng.pdf

Fitz-Gibbon S, Tomida S, Chiu B-H, Nguyen L, Du C, Liu M, Elashoff D, Erfe MC, Loncaric A, Kim J, Modlin RL, Miller JF, Sodergren E, Craft N, Weinstock GM, Li H (2013) Propionibacterium acnes strain populations in the human skin microbiome associated with acne. The Journal of Investigative Dermatology 133(9): 2152–2160. https://doi.org/10.1038/jid.2013.21

Flynn JM, Brown EA, Chain FJJ, MacIsaac HJ, Cristescu ME (2015) Toward accurate molecular identification of species in complex environmental samples: Testing the performance of sequence filtering and clustering methods. Ecology and Evolution 13(11): 2252–2266. https://doi.org/10.1002/ece3.1497

Foote AD, Thomsen PF, Sveegaard S, Wahlberg M, Kielgast J, Kyhn LA, Salling AB, Galatius A, Orlando L, Gilbert MTP (2012) Investigating the potential use of environmental

DNA (eDNA) for genetic monitoring of marine mammals. PLoS ONE 7(8): e41781. https://doi.org/10.1371/journal.pone.0041781

García-Machado E, Normandeaau E, Côté G, Bernatchez L (2023) How eDNA data filtration, sequence coverage, and primer selection influence assessment of fish communities in northern temperate lakes. Environmental DNA 5(6): 1216–1233. https://doi.org/10.1002/edn3.444

Gaydos JK, Leger JS, Raverty S, Nollens H, Haulena M, Ward EJ, Emmons CK, Hanson MB, Balcomb K, Ellifrit D, Weiss MN, Giles D (2023) Epidemiology of skin changes in endangered Southern Resident killer whales (*Orcinus orca*). PLoS ONE 18: e0286551. https://doi.org/10.1371/journal.pone.0286551

Glaeser SP, Silva LMR, Prieto R, Silva MA, Franco A, Kämpfer P, Hermosilla C, Taubert A, Eisenberg T (2022) A preliminary comparison on faecal microbiomes of free-ranging large baleen (*Balaenoptera musculus*, *B. physalus*, *B. borealis*) and toothed (*Physeter macrocephalus*) whales. Microbial Ecology 83(1): 18–33. https://doi.org/10.1007/s00248-021-01729-4

Grice EA, Segre JA (2011) The skin microbiome. Nature Reviews. Microbiology 9(4): 244–253. https://doi.org/10.1038/nrmicro2537

Grimes C, Brent LJN, Weiss MN, Franks DW, Balcomb KC, Ellifrit DK, Ellis S, Croft DP (2022) The effect of age, sex, and resource abundance on patterns of rake markings in resident killer whales (*Orcinus orca*). Marine Mammal Science 38(3): 941–958. https://doi.org/10.1111/mms.12908

Hamilton PK, Marx MK (2005) Skin lesions on North Atlantic right whales: Categories, prevalence and change in occurrence in the 1990s. Diseases of Aquatic Organisms 68: 71–82. https://doi.org/10.3354/dao068071

Herr H, Burkhardt-Holm P, Heyer K, Siebert U, Selling J (2020) Injuries, malformations, and epidermal conditions in cetaceans of the Strait of Gibraltar. Aquatic Mammals 46(2): 215–235. https://doi.org/10.1578/AM.46.2.2020.215

Hicks BD, St. Aubin DJ, Geraci JR, Brown WR (1985) Epidermal growth in the bottlenose dolphin, *Tursiops truncatus*. The Journal of Investigative Dermatology 85: 60–63. https://doi.org/10.1111/1523-1747.ep12275348

Hogg CJ, Rogers TL, Shorter A, Barton K, Miller PJO, Nowacek D (2009) Determination of steroid hormones in whale blow: It is possible. Marine Mammal Science 25(3): 605–618. https://doi.org/10.1111/j.1748-7692.2008.00277.x

Hong J, Karaoz U, de Valpine P, Fithian W (2022) To rarefy or not to rarefy: Robustness and efficiency trade-offs of rarefying microbiome data. Bioinformatics 38(9): 2389–2396. https://doi.org/10.1093/bioinformatics/btac127

Hooker SK, Whitehead H, Gowans S (2002) Ecosystem consideration in conservation planning: Energy demand of foraging bottlenose whales (*Hyperoodon ampullatus*) in a marine protected area. Biological Conservation 104(1): 51–58. https://doi.org/10.1016/S0006-3207(01)00153-7

Hooper R, Brealey JC, van der Valk T, Alberdi A, Durban JW, Fearnbach H, Robertson KM, Baird RW, Bradley Hanson M, Wade P, Gilbert MTP, Morin PA, Wolf JBW, Foote AD, Guschanski K (2019) Host-derived population genomics data provides insights into bacterial and diatom composition of the killer whale skin. Molecular Ecology 28(2): 484–502. https://doi.org/10.1111/mec.14860

Houghton J, Baird RW, Emmons CK, Hanson MB (2015) Changes in the occurrence and behavior of mammal-eating killer whales in southern British Columbia and Washington State, 1987–2010. Northwest Science 89(2): 154–169. https://doi.org/10.3955/046.089.0207

Jeunen G-J, Lipinskaya T, Gajduchenko H, Golovenchik V, Moroz M, Rizevsky V, Semenchenko V, Gemmell NJ (2022) Environmental DNA (eDNA) metabarcoding surveys show evidence of non-indigenous freshwater species invasion to new parts of Eastern Europe. Metabarcoding and Metagenomics 6: e68575. https://doi.org/10.3897/mbmg.6.e68575

Jia B, Garlock E, Allison MJ, Michaud R, Lo R, Round JM, Helbing CC, Verreault J, Brinkman FSL (2022) Investigating the relationship between the skin microbiome and flame retardant exposure of the endangered St. Lawrence Estuary beluga. Frontiers in Environmental Science 10: 954060. https://doi.org/10.3389/fenvs.2022.954060

Kang I, Lim Y, Cho J-C (2018) Complete genome sequence of *Granulosicoccus antarcticus* type strain IMCC3135T, a marine gammaproteobacterium with a putative dimethylsulfoniopropionate demethylase gene. Marine Genomics 37: 176–181. https://doi.org/10.1016/j.margen.2017.11.005

Kaufmann J, Schering A (2014) Analysis of Variance ANOVA. In: Wiley StatsRef: Statistics Reference Online. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118445112.stat06938

Kelly MT, Brock TD (1969) Physiological Ecology of Leucothrix mucor. Journal of General Microbiology 59(2): 153–162. https://doi.org/10.1099/00221287-59-2-153

Keselman HJ, Rogan JC (1977) The Tukey multiple comparison test: 1953–1976. Psychological Bulletin 84(5): 1050–1056. https://doi.org/10.1037/0033-2909.84.5.1050

Kiszka J, Van Bressem M-F, Pusineri C (2009) Lobomycosis-like disease and other skin conditions in Indo-Pacific bottlenose dolphins *Tursiops aduncus* from the Indian Ocean. Diseases of Aquatic Organisms 84: 151–157. https://doi.org/10.3354/dao02037

Koch MS, Silva VDPD, Bracarense APFRL, Domit C (2018) Environmental aspects and diseases related to immunosuppression in cetaceans: A concise review. Veterinary Medicine 39: 2897. https://doi.org/10.5433/1679-0359.2018v39n6p2897

Kolde R (2025) pheatmap: Pretty Heatmaps. https://cran.r-project.org/web/packages/pheatmap/index.html [September 18, 2025]

Lavrador AS, Amaral FG, Moutinho J, Vieira PE, Costa FO, Duarte S (2024) Comprehensive DNA metabarcoding-based detection of non-indigenous invertebrates in recreational marinas through a multi-substrate approach. Marine Environmental Research 200: 106660. https://doi.org/10.1016/j.marenvres.2024.106660

Leadbetter JR (2003) Cultivation of recalcitrant microbes: Cells are alive, well and revealing their secrets in the 21st century laboratory. Current Opinion in Microbiology 6(3): 274–281. https://doi.org/10.1016/S1369-5274(03)00041-9

Lebeer S, Vanderleyden J, De Keersmaecker SCJ (2008) Genes and molecules of lactobacilli supporting probiotic action. Microbiology and molecular biology reviews: MMBR 72: 728–764, Table of Contents. https://doi.org/10.1128/MMBR.00017-08

Leyden JJ, McGiley KJ, Mills OH, Kligman AM (1975) Age-related changes in the resident bacterial flora of the human face. The Journal of Investigative Dermatology 65(4): 379–381. https://doi.org/10.1111/1523-1747.ep12607630

Li J, McLaughlin RW, Chen M, Liu YL, Xie HX, Wan XL, Zhou JY, Zheng JS (2020) First case of Shewanella indica isolated from a Bryde's whale (*Balaenoptera edeni*) stranded in the northern Beibu Gulf, China. Antonie van Leeuwenhoek 113(9): 1385–1391. https://doi.org/10.1007/s10482-020-01444-z

Li C, Xie H, Sun Y, Zeng Y, Tian Z, Chen X, Sanganyado E, Lin J, Yang L, Li P, Liang B, Liu W (2022) Insights on gut and skin wound microbiome in stranded Indo-Pacific finless porpoise (*Neophocaena phocaenoides*). Microorganisms 10(7): 1295. https://doi.org/10.3390/microorganisms10071295

Lindström ES, Langenheder S (2012) Local and regional factors influencing bacterial community assembly. Environmental Microbiology Reports 4(1): 1–9. https://doi.org/10.1111/j.1758-2229.2011.00257.x

Lucas FS, Moureau B, Jourdie V, Heeb P (2005) Brood size modifications affect plumage bacterial assemblages of European starlings. Molecular Ecology 14(2): 639–646. https://doi.org/10.1111/j.1365-294X.2005.02436.x

Maćkiewicz A, Ratajczak W (1993) Principal components analysis (PCA). Computers & Geosciences 19(3): 303–342. https://doi.org/10.1016/0098-3004(93)90090-R

Maldini D, Riggin J, Cecchetti A, Cotter MP (2010) Prevalence of epidermal conditions in California coastal bottlenose dolphins (*Tursiops truncatus*) in Monterey Bay. Ambio 39(7): 455–462. https://doi.org/10.1007/s13280-010-0066-8

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.Journal 17(1): 10–12. https://doi.org/10.14806/ej.17.1.200

Martineau D (2007) Potential synergism between stress and contaminants in free-ranging cetaceans. International Journal of Comparative Psychology 20(2): 194–216. https://doi.org/10.46867/IJCP.2007.20.02.06

Mathews EA, Keller S, Weiner DB (1988) A method to collect and process skin biopsies for cell culture from free-ranging gray whales (*Eschrichtius robustus*). Marine Mammal Science 4(1): 1–12. https://doi.org/10.1111/j.1748-7692.1988.tb00178.x

McMurdie P, Holmes S (2014) Waste not, want not: Why rarefying microbiome data is inadmissible. PLoS Computational Biology 10(4): e1003531. https://doi.org/10.1371/journal.pcbi.1003531

Milián-García Y, Pyne C, Lindsay K, Romero A, Hanner RH (2023) Unveiling invasive insect threats to plant biodiversity: Leveraging eDNA metabarcoding and saturated salt trap solutions for biosurveillance. PLoS ONE 18(8): e0290036. https://doi.org/10.1371/journal.pone.0290036

Morin PA, Parsons KM, Archer FI, Ávila-Arcos MC, Barrett-Lennard LG, Dalla Rosa L, Duchêne S, Durban JW, Ellis GM, Ferguson SH, Ford JK, Ford MJ, Garilao C, Gilbert MTP, Kaschner K, Matkin CO, Petersen SD, Robertson KM, Visser IN, Wade PR, Ho SYW, Foote AD (2015) Geographic and temporal dynamics of a global radiation and diversification in the killer whale. Molecular Ecology 24(15): 3964–3979. https://doi.org/10.1111/mec.13284

Morin PA, McCarthy ML, Fung CW, Durban JW, Parsons KM, Perrin WF, Taylor BL, Jefferson TA, Archer FI (2024) Revised taxonomy of eastern North Pacific killer whales (*Orcinus orca*): Bigg's and resident ecotypes deserve species status. Royal Society Open Science 11(3): 231368. https://doi.org/10.1098/rsos.231368

Mouton M, Botha A (2012) Cutaneous Lesions in Cetaceans: An Indicator of Ecosystem Status? In: New Approaches to the Study of Marine Mammals. BoD – Books on Demand, 123–150. https://doi.org/10.5772/54432

Mueller RS, Bryson S, Kieft B, Li Z, Pett-Ridge J, Chavez F, Hettich RL, Pan C, Mayali X (2015) Metagenome sequencing of a coastal marine microbial community from Monterey Bay, California. Genome Announcements 3: 10.1128/genomea.00341-15. https://doi.org/10.1128/genomea.00341-15

Murphy CJ, Collier MA, Jacoby A-M, Patterson EM, Wallen MM, Mann J, Bansal S (2025) Automated skin lesion detection and prevalence estimation in Tamanend's bottlenose dolphins. Ecological Informatics 85: 102942. https://doi.org/10.1016/j.ecoinf.2024.102942

Ndongo S, Lagier J-C, Fournier P-E, Raoult D, Khelaifia S (2016) "*Prevotellamassilia timonensis*," a new bacterial species isolated from the human gut. New Microbes and New Infections 5: 102–103. https://doi.org/10.1016/j.nmni.2016.06.014

Needham DM, Chow C-ET, Cram JA, Sachdeva R, Parada A, Fuhrman JA (2013) Short-term observations of marine bacterial and viral communities: Patterns, connections and resilience. The ISME Journal 7(7): 1274–1285. https://doi.org/10.1038/ismej.2013.19

Nicholls CR, Peters KJ, Cagnazzi D, Hanf D, Parra GJ (2023) Incidence of shark-inflicted bite injuries on Australian snubfin (*Orcaella heinsohni*) and Australian humpback (*Sousa sahulensis*) dolphins in coastal waters off east Queensland, Australia. Ecology and Evolution 13(5): e10026. https://doi.org/10.1002/ece3.10026

Nisi AC, Welch H, Brodie S, Leiphardt C, Rhodes R, Hazen EL, Redfern JV, Branch TA, Barreto AS, Calambokidis J, Clavelle T, Dares L, de Vos A, Gero S, Jackson JA, Kenney RD, Kroodsma D, Leaper R, McCauley DJ, Moore SE, Ovsyanikova E, Panigada S, Robinson CV, White T, Wilson J, Abrahms B (2024) Ship collision risk threatens whales across the world's oceans. Science 386(6724): 870–875. https://doi.org/10.1126/science.adp1950

Noren DP, Mocklin JA (2012) Review of cetacean biopsy techniques: Factors contributing to successful sample collection and physiological and behavioral impacts. Marine Mammal Science 28(1): 154–199. https://doi.org/10.1111/j.1748-7692.2011.00469.x

O'Mahony ÉN, Sremba AL, Keen EM, Robinson N, Dundas A, Steel D, Wray J, Baker CS, Gaggiotti OE (2024) Collecting baleen whale blow samples by drone: A minimally intrusive tool for conservation genetics. Molecular Ecology Resources 24(8): e13957. https://doi.org/10.1111/1755-0998.13957

Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Solymos P, Stevens MHH, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista HBA, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill MO, Lahti L, McGlinn D, Ouellette M-H, Ribeiro Cunha E, Smith T, Stier A, Ter Braak CJF, Weedon J, Borman T (2001) vegan. Community Ecology Package. 2: 6–10. https://doi.org/10.32614/CRAN.package.vegan

Parsons KM, Everett M, Dahlheim M, Park L (2018) Water, water everywhere: Environmental DNA can unlock population structure in elusive marine species. Royal Society Open Science 5(8): 180537. https://doi.org/10.1098/rsos.180537

Parsons KM, May SA, Gold Z, Dahlheim M, Gabriele C, Straley JM, Moran JR, Goetz K, Zerbini AN, Park L, Morin PA (2024) Using eDNA to Supplement Population Genetic Analyses for Cryptic Marine Species: Identifying population boundaries for Alaska harbour porpoises. Molecular Ecology 34(5): e17563. https://doi.org/10.1111/mec.17563

Pascoal F, Tomasino MP, Piredda R, Quero GM, Torgo L, Poulain J, Galand PE, Fuhrman JA, Mitchell A, Tinta T, Turk Dermastia T, Fernandez-Guerra A, Vezzi A, Logares R, Malfatti F, Endo H, Dąbrowska AM, De Pascale F, Sánchez P, Henry N, Fosso B, Wilson B, Toshchakov S, Ferrant GK, Grigorov I, Vieira FRJ, Costa R, Pesant S, Magalhães C (2023) Inter-comparison of marine microbiome sampling protocols. ISME Communications 3(1): 84. https://doi.org/10.1038/s43705-023-00278-w

Perez Perez GI, Gao Z, Jourdain R, Ramirez J, Gany F, Clavaud C, Demaude J, Breton L, Blaser MJ (2016) Body site is a more determinant factor than human population Diversity in the healthy skin microbiome. PLoS ONE 11(4): e0151990. https://doi.org/10.1371/journal.pone.0151990

Pirotta V, Smith A, Ostrowski M, Russell D, Jonsen ID, Grech A, Harcourt R (2017) An economical custom-built drone for assessing whale health. Frontiers in Marine Science 4: 425. https://doi.org/10.3389/fmars.2017.00425

Pitman RL, Durban JW, Joyce T, Fearnbach H, Panigada S, Lauriano G (2020) Skin in the game: Epidermal molt as a driver of long-distance migration in whales. Marine Mammal Science 36(2): 565–594. https://doi.org/10.1111/mms.12661

Porter TM, Hajibabaei M (2022) MetaWorks: A flexible, scalable bioinformatic pipeline for high-throughput multi-marker biodiversity assessments. PLoS ONE 17(9): e0274260. https://doi.org/10.1371/journal.pone.0274260

Qiagen (2020) DNeasy® Blood & Tissue Handbook. http://www.bea.ki.se/documents/EN-DNeasy%20handbook.pdf

Qin W, Martens-Habbena W, Kobelt JN, Stahl DA (2016) Candidatus Nitrosopumilaceae. In: Bergey's Manual of Systematics of Archaea and Bacteria. John Wiley & Sons, Ltd, 1–2. https://doi.org/10.1002/9781118960608.fbm00262

R Core Team (2024) R: A Language and Environment for Statistical Computing. https://www.R-project.org/

Raverty SA, Gaydos JK, St. Leger J (2014) Killer whale necropsy and disease testing protocol, 82 pp. https://static1.squarespace.com/static/5b071ddea2772cebc1662831/t/5b29e3bb352f539b40739ecd/1529471951000/Orca-necropsy-protocol-FINAL-May-15-2014.pdf

Raverty SA, Rhodes LD, Zabek E, Eshghi A, Cameron CE, Hanson MB, Schroeder JP (2017) Respiratory microbiome of endangered Southern Resident killer whales and microbiota of surrounding sea surface microlayer in the eastern North Pacific. Scientific Reports 7(1): 394. https://doi.org/10.1038/s41598-017-00457-5

Raverty S, Leger JS, Noren DP, Huntington KB, Rotstein DS, Gulland FMD, Ford JKB, Hanson MB, Lambourn DM, Huggins J, Delaney MA, Spaven L, Rowles T, Barre L, Cottrell P, Ellis G, Goldstein T, Terio K, Duffield D, Rice J, Gaydos JK (2020) Pathology findings and correlation with body condition index in stranded killer whales (*Orcinus orca*) in the northeastern Pacific and Hawaii from 2004 to 2013. PLoS ONE 15: e0242505. https://doi.org/10.1371/journal.pone.0242505

Reji L, Tolar BB, Chavez FP, Francis CA (2020) Depth-differentiation and seasonality of planktonic microbial assemblages in the Monterey Bay upwelling system. Frontiers in Microbiology 11: 1075. https://doi.org/10.3389/fmicb.2020.01075

Rhodes LD, Emmons CK, Wisswaesser G, Wells AH, Hanson MB (2022) Bacterial microbiomes from mucus and breath of southern resident killer whales (*Orcinus orca*). Conservation Physiology 10(1): coac014. https://doi.org/10.1093/conphys/coac014

Rhodes LD, Adams NG, Gallego Simon R, Kavanaugh MT, Alin SR, Feely RA (2024) Nearshore microbial communities of the Pacific Northwest coasts of Canada and the U.S. Frontiers in Marine Science 11: 1430930. https://doi.org/10.3389/fmars.2024.1430930

Robeck TR, St. Leger JA, Robeck HE, Nilson E, Dold C (2019) Evidence of variable agonistic behavior in killer whales (*Orcinus orca*) based on age, sex, and ecotype. Aquatic Mammals 45: 430–446. https://doi.org/10.1578/AM.45.4.2019.430

Robinson CV, Nuuttila HK (2020) Don't hold your breath: Limited DNA capture using non-invasive blow sampling for small cetaceans. Aquatic Mammals 46(1): 32–41. https://doi.org/10.1578/AM.46.1.2020.32

Robinson CV, Porter TM, Maitland VC, Wright MT, Hajibabaei M (2022) Multi-marker metabarcoding resolves subtle variations in freshwater condition: Bioindicators, ecological traits, and trophic interactions. Ecological Indicators 145: 109603. https://doi.org/10.1016/j.ecolind.2022.109603

Robinson CV, Dracott K, Glover RD, Warner A, Migneault A (2024) DNA from dives: Species detection of humpback whales (Megaptera novaeangliae) from flukeprint eDNA. Environmental DNA 6(2): e524. https://doi.org/10.1002/edn3.524

Robinson CV, Laqua E, Migneault A, Sutton GJ, Dracott K, Bachert A (2025a) Gone in a splash? Temporal dynamics of flukeprint environmental DNA (eDNA) detection

for common coastal northeast Pacific cetacean species. Environmental DNA 7(3): e70132. https://doi.org/10.1002/edn3.70132

Robinson CV, Laqua EJ, Warner A, Sutton GJ, Judson MWD, Dracott K (2025b) Sexing from seawater: Application of environmental DNA beyond species detection for cetaceans. Environmental DNA 7(4): e70173. https://doi.org/10.1002/edn3.70173

Robles-Malagamba MJ, Walsh MT, Ahasan MS, Thompson P, Wells RS, Jobin C, Fodor AA, Winglee K, Waltzek TB (2020) Characterization of the bacterial microbiome among free-ranging bottlenose dolphins (*Tursiops truncatus*). Heliyon 6(6): e03944. https://doi.org/10.1016/j.heliyon.2020.e03944

Rodriguez LK, Bonis LD, McKee J, McKenna JA, Urvois T, Barbaccia E, Dillane E, Lanfredi C, Hjellnes H, Jung A, Villa E, Azzellino A, Westgaard J-I, Quéméré E, Thalinger B (2025) Inter-laboratory ring test for environmental DNA extraction protocols: Implications for marine megafauna detection using three novel qPCR assays. Metabarcoding and Metagenomics 9: e128235. https://doi.org/10.3897/mbmg.9.128235

Rognes T, Flouri T, Nichols B, Quince C, Mahé F (2016) VSEARCH: A versatile open source tool for metagenomics. PeerJ 4: e2584. https://doi.org/10.7717/peerj.2584

Ross PS (2002) The role of immunotoxic environmental contaminants in facilitating the emergence of infectious diseases in marine mammals. Human and Ecological Risk Assessment 8(2): 277–292. https://doi.org/10.1080/20028091056917

Ross AA, Müller KM, Weese JS, Neufeld JD (2018) Comprehensive skin microbiome analysis reveals the uniqueness of human skin and evidence for phylosymbiosis within the class Mammalia. Proceedings of the National Academy of Sciences of the United States of America 115(25): E5786–E5795. https://doi.org/10.1073/pnas.1801302115

RStudio (2023) RStudio: Integrated Development Environment for R. http://www.rstudio.com/

Santos B, Afonso L, Alves F, Dinis A, Ferreira R, Correia AM, Valente R, Gil Á, Castro LFC, Sousa-Pinto I, Rosso M, Centelleghe C, Mazzariol S, Magalhães C, Tomasino MP (2024) Hidden in the blow - a matrix to characterise cetaceans' respiratory microbiome: Short-finned pilot whale as case study. Metabarcoding and Metagenomics 8: e121060. https://doi.org/10.3897/mbmg.8.121060

Schloerke B, Cook D, Larmarange J, Briatte F, Marbach M, Thoen E, Elberg A, Toomet O, Crowley J, Hofmann H, Wickham H (2024) GGally: Extension to "ggplot2." https://cran.r-project.org/web/packages/GGally/index.html [June 21, 2025]

Schloss PD (2024) Rarefaction is currently the best approach to control for uneven sequencing effort in amplicon sequence analyses. MSphere 9(2): e0035423. https://doi.org/10.1128/msphere.00354-23

Sehnal L, Brammer-Robbins E, Wormington AM, Blaha L, Bisesi J, Larkin I, Martyniuk CJ, Simonin M, Adamovsky O (2021) Microbiome composition and function in aquatic vertebrates: Small organisms making big impacts on aquatic animal health. Frontiers in Microbiology 12: 567408. https://doi.org/10.3389/fmicb.2021.567408

Sevellec M, Lacoursière-Roussel A, Normandeau E, Bernatchez L, Lynn Howland K (2025) Effect of eDNA metabarcoding temporal sampling strategies on detection of coastal biodiversity. Frontiers in Marine Science 12: 1522677. https://doi.org/10.3389/fmars.2025.1522677

Shapiro S, Wilk M (1965) An analysis of variance test for normality (complete samples). Biometrika 52(3–4): 591–611. https://doi.org/10.1093/biomet/52.3-4.591

Shenhav L, Thompson M, Joseph TA, Briscoe L, Furman O, Bogumil D, Mizrahi I, Pe'er I, Halperin E (2019) FEAST: Fast expectation-maximization for microbial source tracking. Nature Methods 16(7): 627–632. https://doi.org/10.1038/s41592-019-0431-x

Shields MW, Hysong-Shimazu S, Shields JC, Woodruff J (2018) Increased presence of mammal-eating killer whales in the Salish Sea with implications for predator-prey dynamics. PeerJ 6: e6062. https://doi.org/10.7717/peerj.6062

Shirazi S, Meyer RS, Shapiro B (2021) Revisiting the effect of PCR replication and sequencing depth on biodiversity metrics in environmental DNA metabarcoding. Ecology and Evolution 11(22): 15766–15779. https://doi.org/10.1002/ece3.8239

Small C, Nicholls R (2003) A global analysis of human settlement in coastal zones. Journal of Coastal Research 19: 584–599. https://www.jstor.org/stable/4299200

Sobell MG (2011) A Practical Guide to Ubuntu Linux. Pearson Education, 1307 pp.

St John J (2016) SeqPrep. https://github.com/jstjohn/SeqPrep/releases

Suarez-Bregua P, Álvarez-González M, Parsons KM, Rotllant J, Pierce GJ, Saavedra C (2022) Environmental DNA (eDNA) for monitoring marine mammals: Challenges and opportunities. Frontiers in Marine Science 9: 987774. https://doi.org/10.3389/fmars.2022.987774

Sun X, Kop LFM, Lau MCY, Frank J, Jayakumar A, Lücker S, Ward BB (2019) Uncultured Nitrospina-like species are major nitrite oxidizing bacteria in oxygen minimum zones. The ISME Journal 13(10): 2391–2402. https://doi.org/10.1038/s41396-019-0443-7

Sun S, Jones RB, Fodor AA (2020) Inference-based accuracy of metagenome prediction tools varies across sample types and functional categories. Microbiome 8(1): 46. https://doi.org/10.1186/s40168-020-00815-y

Székely D, Corfixen NL, Mørch LL, Knudsen SW, McCarthy ML, Teilmann J, Heide-Jørgensen MP, Olsen MT (2021) Environmental DNA captures the genetic diversity of bowhead whales (*Balaena mysticetus*) in West Greenland. Environmental DNA 3(1): 248–260. https://doi.org/10.1002/edn3.176

Tessler M, Brugler MR, DeSalle R, Hersch R, Velho LFM, Segovia BT, Lansac-Toha FA, Lemke TJ (2017) A global eDNA comparison of freshwater bacterioplankton assemblages focusing on large-river floodplain lakes of Brazil. Microbial Ecology 73(1): 61–74. https://doi.org/10.1007/s00248-016-0834-5

Toro F, Alarcón J, Márquez S, Capella J, Bahamonde P, Esperón F, Moreno-Switt A, Castro-Nallar E (2021) Composition and structure of the skin microbiota of rorquals off the Eastern South Pacific. FEMS Microbiology Ecology 97(5): fiab050. https://doi.org/10.1093/femsec/fiab050

Toro Cortes F (2019) The frontline: assessment of the skin bacterial communities of three species of rorquals from different environments with human impacts. Universidad Andrés Bello. https://repositorio.unab.cl/xmlui/handle/ria/54562 [June 21, 2025]

Towers J, Sutton G, Shaw T, Malleson M, Matkin D, Gisborne B, Forde J, Ellifrit D, Ellis G, Ford J, Doniol-Valcroze T (2019) Photo-identification catalogue, population status, and distribution of Bigg's killer whales known from coastal waters of British Columbia, Canada, [vi +] 299 pp.

Ul-Hasan S, Bowers RM, Figueroa-Montiel A, Licea-Navarro AF, Beman JM, Woyke T, Nobile CJ (2019) Community ecology across bacteria, archaea and microbial eukaryotes in the sediment and seawater of coastal Puerto Nuevo, Baja California. PLoS ONE 14(2): e0212355. https://doi.org/10.1371/journal.pone.0212355

Van Cise AM, Wade PR, Goertz CEC, Burek-Huntington K, Parsons KM, Clauss T, Hobbs RC, Apprill A (2020) Skin microbiome of beluga whales: Spatial, temporal, and

health-related dynamics. Animal Microbiome 2(1): 39. https://doi.org/10.1186/s42523-020-00057-1

van Weelden C, Tixier P, Doniol-Valcroze T, Guinet C, Towers JR (2025) Divergent killer whale populations exhibit similar acquisition but different healing rates of conspecific scars. Behavioral Ecology and Sociobiology 79(3): 39. https://doi.org/10.1007/s00265-025-03576-6

Vendl C, Slavich E, Wemheuer B, Nelson T, Ferrari B, Thomas T, Rogers T (2020) Respiratory microbiota of humpback whales may be reduced in diversity and richness the longer they fast. Scientific Reports 10(1): 12645. https://doi.org/10.1038/s41598-020-69602-x

von Lampe F, Schellenberg J (2024) goeveg: Functions for Community Data and Ordinations. https://cran.r-project.org/web/packages/goeveg/index.html [January 31, 2025]

Wade PR, Forney KA (2007) Worldwide Distribution And Abundance Of Killer Whales. In: Estes JA, DeMaster DP, Doak DF, Williams TM, Brownell RL (Eds) Whales, Whaling, and Ocean Ecosystems. University of California Press, 145–162. https://doi.org/10.1525/9780520933200-016

Wang Q, Cole JR (2024) Updated RDP taxonomy and RDP Classifier for more accurate taxonomic classification. Microbiology Resource Announcements 13(4): e01063–e23. https://doi.org/10.1128/mra.01063-23

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian Classifier for Rapid Assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73(16): 5261–5267. https://doi.org/10.1128/AEM.00062-07

Weber L, Apprill A (2020) Diel, daily, and spatial variation of coral reef seawater microbial communities. PLoS ONE 15(3): e0229442. https://doi.org/10.1371/journal.pone.0229442

Weiss S, Xu ZZ, Peddada S, Amir A, Bittinger K, Gonzalez A, Lozupone C, Zaneveld JR, Vázquez-Baeza Y, Birmingham A, Hyde ER, Knight R (2017) Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 5(1): 27. https://doi.org/10.1186/s40168-017-0237-y

Wickham H, Chang W, Henry L, Pedersen TL, Takahashi K, Wilke C, Woo K, Yutani H, Dunnington D, van den Brand T, Posit PBC (2024) ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics. https://cran.r-project.org/web/packages/ggplot2/index.html [December 9, 2024]

Xu L, Wu Y-H, Jian S-L, Wang C-S, Wu M, Cheng L, Xu X-W (2016) Pseudohongiellanitratireducens sp. nov., isolated from seawater, and emended description of the genus Pseudohongiella. International Journal of Systematic and Evolutionary Microbiology 66(12): 5155–5160. https://doi.org/10.1099/ijsem.0.001489

Yang S-H, Seo H-S, Woo J-H, Oh H-M, Jang H, Lee J-H, Kim S-J, Kwon KK (2014) Carboxylicivirga gen. nov. in the family Marinilabiliaceae with two novel species, Carboxylicivirga mesophila sp. nov. and Carboxylicivirga taeanensis sp. nov., and reclassification of Cytophaga fermentans as Saccharicrinis fermentans gen. nov., comb. nov. International Journal of Systematic and Evolutionary Microbiology 64(Pt_4): 1351–1358. https://doi.org/10.1099/ijs.0.053462-0

Zhang J, Wang X, Huo D, Li W, Hu Q, Xu C, Liu S, Li C (2016) Metagenomic approach reveals microbial diversity and predictive microbial metabolic pathways in Yucha, a traditional Li fermented food. Scientific Reports 6(1): 32524. https://doi.org/10.1038/srep32524

Zhang S, Amanze C, Sun C, Zou K, Fu S, Deng Y, Liu X, Liang Y (2021) Evolutionary, genomic, and biogeographic characterization of two novel xenobiotics-degrading strains affiliated with *Dechloromonas*. Heliyon 7(6): e07181. https://doi.org/10.1016/j.heliyon.2021.e07181

Zinger L, Amaral-Zettler LA, Fuhrman JA, Horner-Devine MC, Huse SM, Welch DBM, Martiny JBH, Sogin M, Boetius A, Ramette A (2011) Global Patterns of Bacterial Beta-Diversity in Seafloor and Seawater Ecosystems. PLoS ONE 6(9): e24570. https://doi.org/10.1371/journal.pone.0024570

Zorz J, Willis C, Comeau AM, Langille MGI, Johnson CL, Li WKW, LaRoche J (2019) Drivers of Regional Bacterial Community Structure and Diversity in the Northwest Atlantic Ocean. Frontiers in Microbiology 10: 281. https://doi.org/10.3389/fmicb.2019.00281

Supplementary material 1

Supplementary information

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Data type: docx

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